Control of Typhoid Fever: Evaluating Herd Protection through Public Health Use of Typhoid Vi Polysaccharide Vaccine

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Abstract

Typhoid fever remains an important public health problem globally. Cluster randomized effectiveness trials with typhoid Vi polysaccharide vaccine were conducted in Kolkata, India and Karachi, Pakistan, to provide evidence for vaccine introduction. While efficacy trials are limited to estimate vaccine's performance on the vaccine recipients, effectiveness trials consider the public health impact, notably the herd protection, or indirect effect, which can only be seen when vaccines are administered to groups rather than to individuals.

The observed total protection by the Vi polysaccharide vaccine in school-aged children was consistent in Kolkata and Karachi (61% and 56%, respectively), and was associated with minimal side-effects. The total protection in young children, however, was different (80% in Kolkata and no protection in Karachi). The Kolkata trial demonstrated significant herd protective effects, as demonstrated by indirect protection of non-vaccinees (45%), which was not shown in the Karachi trial. The difference in the effectiveness estimates between the trials may be due to the difference in study design and the population characteristics.

Immunogenicity studies were undertaken for randomly selected persons from both sites at pre-vaccination, 6 weeks, and 2 years post-vaccination. Serum Vi antibody titres (IgG) were measured through ELISA. At baseline, the GMTs were below the protective level for both sites. At six weeks after vaccination, though there is a significant increase in the GMTs in children from both site, the level of GMTs were significantly lower from those in Karachi (2,307.0 ELU vs. 1,189.1 ELU). GMT declined from 6 week to 2 year testing points for both sites but maintained the protective level.

These effectiveness trials gave a conclusive evidence of the protection conferred by the Vi polysaccharide vaccine in children older than 5 years of age. Targeted vaccination programme in high endemic areas, as stipulated in the WHO Position Paper, suggest the potential for effective control of typhoid fever in places like India and Pakistan with the school-based Vi vaccination.

Contributors

This thesis comprises of work conducted through the Diseases of the Most Impoverished (DOMI) Programme, funded by the Bill and Melinda Gates Foundation and administered by the International Vaccine Institute (IVI). The studies have started before my enrolment to the Doctor of Philosophy curriculum; however, I have led from the IVI's side to develop the study site, develop the Standard Operating Procedures, regular monitoring and supervision, and the final analyses, which is hereby presented as my work during the curriculum.

* * *

The Programme Principal Investigator was Dr. John D. Clemens, Director General of the IVI, while the site Principal Investigators were Dr. Sujit K. Bhattacharya, Director of the National Institute of Cholera and Enteric Diseases (NICED) in Kolkata and Prof. Zulfiqar A. Bhutta, Chairman of the Department of Maternal Child Health, Aga Khan University (AKU) in Karachi.

Initially as DOMI Programme Fellow, my responsibilities were to set up the study sites for the typhoid effectiveness trials. Eventually, my role became coordinator for the Typhoid program component of the DOMI Programme as Research Scientist at IVI. It was my responsibility to manage the study sites and to ensure the data validity. This includes enforcing the randomization, vaccination, surveillance, site laboratory management, and site data management. The data, after being cleaned by the local data management team, was again checked for any errors by me together with the IVI's data management team. Analytical plans for these studies have been developed by a group which includes consultant statistician, IVI's statisticians, and IVI's epidemiologists. I was responsible to lead this development of the analytical plan, as well as review of the outputs for any possible errors.

* * *

The effectiveness trial in Kolkata was organized and managed locally by the National Institute of Cholera and Enteric Diseases (NICED). In addition to the said PIs (Dr. J. D. Clemens and Dr. S. K. Bhattacharya), the following persons contributed in the various aspects of the study:

- Dr. Camilo J. Acosta, then Research Scientist, IVI – Overall management of the DOMI Typhoid Programme
- Dr. Remon Abu-Elyazeed, GlaxoSmithKline, Singapore – Study protocol development assistance
- Dr. Mohammad Ali, Senior Scientist, IVI – Data management and analysis
- Ms. Barnali Bhaduri, Data Manager, NICED – Data management
- Dr. Hans Bock, GlaxoSmithKline, Singapore – vaccine and serological test assistance
- Mr. James Campbell, Scientist, OUCRU – Reference laboratory
- Prof. Allan Donner, Professor, University of West Ontario, London, Canada – Data analysis
- Dr. Jacqueline L. Deen, then Research Scientist, IVI – Field site development and management
- Dr. Dharitri Dutta, Scientist, NICED – Clinical supervision
- Dr. Shanta Dutta, Deputy Director, NICED – Laboratory work
- Dr. Claudia M. Galindo, then Research Scientist, IVI – Overall management of the DOMI Typhoid Programme
- Dr. Jeremy Farrar, Director, OUCRU – Reference laboratory
- Dr. Nirmal K. Ganguly, Director, Indian Council of Medical Research, Delhi, India – Study conception
- Dr. Bernard Ivanoff, WHO – Member of DSMB and Study protocol development assistance
- Dr. Luis Jodar, then Deputy Director General, IVI – Overall management
- Dr. Suman Kanungo, Scientist, NICED – Overall supervision
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- Dr. Byomkesh Manna, Deputy Director, NICED – Data management and overall supervision
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- Dr. Tikki Pang, WHO – Study protocol development assistance
- Dr. Jin Kyung Park, Scientific Research Analyst, IVI – Data management and analysis
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- Dr. Lorenz von Seidlein, then Senior Research Scientist, IVI – Overall management of the DOMI Programme
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- Dr. John Wain, then DOMI laboratory task force, IVI – Field site laboratory development and standardization
- Dr. Xu Zhi-yi, then Senior Scientist, IVI – Study protocol development assistance

* * *

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- Dr. Remon Abu-Elyazeed, GlaxoSmithKline, Singapore – Study protocol development assistance
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- Prof. Rumina Hassan, Professor, AKU – Laboratory work
- Dr. Bernard Ivanoff, WHO – Member of DSMB and Study protocol development assistance
- Dr. Luis Jodar, then Deputy Director General, IVI – Overall management
- Dr. Mohammad Imran Khan, Research Supervisor, AKU – Overall supervision
- Ms. Deok Ryun Kim, Statistical Analyst, IVI – Data analysis
- Dr. Dong Wook Kim, Scientist, IVI – Confirmation of the strains
- Prof. S. Qamaruddin Nizami, Professor, AKU – Overall supervision
- Dr. Anne-Laure Page, then DOMI Programme Intern, IVI – Field site laboratory development and standardization
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- Prof. Afia Zafar, Assistant Professor, AKU – Laboratory work
- Dr. Xu Zhi-yi, then Senior Scientist, IVI – Study protocol development assistance

* * *

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In these large trials, the utmost importance is the participation of the community. Though not scientifically involved, the studies were not possible without the assistance and participation of the local community leaders from each site.

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Analytical plans submitted to the Data and Safety Monitoring Board (DSMB) of the Vi effectiveness trials are included in the thesis as appendices.

1. Analytical Plan of the Vi Effectiveness Trials – Kolkata, India
2. Analytical Plan of the Vi Effectiveness Trials – Karachi, Pakistan

List of Publications and Presentations

The publications and presentations from the student period (Michaelmas 2007 to Trinity 2011) are listed as a reference below.

Co-authored Book Chapter

1. Bhutta ZA, Khan MI, Soofi SB, Ochiai RL. **New Advances in Typhoid Fever Vaccination Strategies.** "Hot Topics in Infection and Immunity in Children VII" Editors: Nigel Curtis, Adam Finn, Andrew J. Pollard. Series: Advances in Experimental Medicine and Biology, Vol. 697. Springer Publishing, New York 2010

Authored/Co-authored Published Manuscripts

1. Zhou WZ, Koo HW, Wang XY, Zhang J, Park JK, Zhu F, Deen J, Acosta CJ, Chen Y, Wang H, Galindo CM, Ochiai L, Park T, von Seidlein L, Xu ZY, Clemens JD. **Revaccination with Locally-Produced Vi Typhoid Polysaccharide Vaccine Among Chinese School-Aged Children: Safety and Immunogenicity Findings.** Paediatric Infectious Diseases Journal. November 2007;26(11):1001-1005
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Professional Presentations

1. **"A multicenter, population-based, prospective surveillance study of typhoid fever in 5 Asian countries: disease burden and implications for control"** US-Japan Joint Panels on Cholera and Other Bacterial Enteric Infections; Organized by US-Japan Cooperative Medical Sciences Programme, December 2007, Austin, TX, USA
2. **"Typhoid Vaccines: Current availability, programmatic issues, costs, and vaccine pipeline"** EPI Staff Training Course; Organized by World Health Organization, Western Pacific Regional Office, April 2008, Antipolo City, Philippines
3. **"Vaccine trials, a different challenge for diagnostics"** Continuing Medical Education: Recent advances in diagnosis of Enteric fever; Organized by UK HPA Laboratory of Gastrointestinal Infections, Safdarjung Hospital and Vardhman Mahavir Medical College, and BLK Hospital, November 2008, New Delhi, India
4. **"The DOMI experience: Lessons learned from a 5-country, standardized disease burden study of typhoid in Asia"** Meeting on Formation of a Consortium on Invasive Bacterial Disease Burden with Focus on Invasive Salmonelloses in Sub-Saharan Africa; Organized by International Vaccine Institute and Kenya Medical Research Institute, January 2009, Kilifi, Kenya
5. **"A Phase IV Cluster-Randomized Effectiveness Trial of Vi Polysaccharide Vaccine against Typhoid Fever in Kolkata, India"** 7th International Conference of Typhoid Fever and Other Invasive Salmonelloses; Organized by Kenya Medical Research Institute and World Health Organization, January 2009, Kilifi, Kenya
6. **"Burden of typhoid fever: Global estimates and results of DOMI surveillance and economic studies in five Asian countries" "Typhoid vaccines: Currently available new-generation vaccines and new vaccines in pipeline"** Meeting on Typhoid Fever Vaccination in the Asia-Pacific Regions; Organized by International Vaccine Institute, March 2009, Bangkok, Thailand

7. **"Epidemiology of typhoid, a case for addressing a neglected disease that predominantly affect the disadvantaged"** Regional Workshop on Vaccines Prioritization; Organized by World Health Organization, South-East Asia Regional Office, May 2009, Bangkok, Thailand
8. **"Using Cluster-Randomized Trial Design to Determine Herd Protection with Vi Typhoid Polysaccharide Vaccine – Kolkata, India"** The 5th TEPHINET Southeast Asia and Western Pacific Bi-Regional Scientific Conference; Organized by TEPHINET and Korea Centres for Disease Control (KCDC), November 2009, Seoul, Korea
9. **"Can Typhoid be Eliminated?"** BIT Life Sciences' 2nd World Congress of Vaccine, Mar 2010, Beijing, China
10. **"Vaccination Against Typhoid: feasibility of control of typhoid in high risk areas"** Regional Review Meeting on Immunization; Organized by the World Health Organization Regional Office for South-East Asia, July 2010, Delhi, India
11. **"Typhoid Fever in Five Asian Countries: Disease Burden and Implications for Control"** National Workshop on Control of Typhoid in Fiji; Organized by the World Health Organization South Pacific Office, August 2010, Suva, Fiji
12. **"On Typhoid Control and Prevention"** American Society of Tropical Medicine and Health (ASTMH) annual meeting; Organized by ASTMH, November 2010, Atlanta, GA, USA
13. **"Need for vaccination information: Formative research results of Vi PS typhoid vaccine introduction in Karachi, Pakistan"** American Public Health Association (APHA) annual meeting; Organized by APHA, November 2010, Denver, CO, USA
14. **"Global and regional burden of typhoid disease" "Non-vaccine prevention of typhoid fever"** New Vaccines Meeting; Organized by Government of Nepal and NCIP, December 2010, Kathmandu, Nepal
15. **"Global Typhoid Disease Burden and Challenges of Typhoid Surveillance" "Use of Typhoid Vaccine: Experience from DOMI Project and the Feasibility of Typhoid Vaccine Use in Public Health Programs"** Ad-hoc Consultation on Typhoid Vaccine Introduction and Typhoid Surveillance; Organized by the World Health Organization, April 2011, Bangkok, Thailand

1 INTRODUCTION

1.1 Epidemiology

Typhoid fever, a systemic illness caused by infection from *Salmonella enterica* serovar serotype Typhi (*S.* Typhi), remains an important public health problem in the world especially in the impoverished populations of developing countries. *S.* Typhi is transmitted via the oral-faecal route through contaminated water or food. The disease is usually associated with poor sanitation and hygiene practices. Interestingly, the disease was rampant in the United States of America (US) and Europe until relatively recently, especially in the densely populated urban areas. It was not so long ago, during the turn of 20th Century in urban New York, when Mary Mallon was unknowingly infecting others with typhoid and indirectly killing them, eventually receiving the notorious name “Typhoid Mary”. The disease may no longer be so prevalent now but it is still present in developed countries. Although the majority of reported and identified cases are associated with travels to typhoid endemic countries, there are also reported indigenous transmissions of typhoid fever in the US and in other developed countries (Hoshino, Masuda et al. 2000; Cooke, Day et al. 2007; Lynch, Blanton et al. 2009; Patel, Armstrong et al. 2010). About 19 percent of US typhoid cases were associated with local outbreaks such as the one in New York in 1998 that involved 47 people, which was attributed to drinking orange juice contaminated by a food handler (Mermin, Townes et al. 1998). Furthermore, recently, a typhoid fever outbreak associated with fruit bars was major news in some parts of the United States where 9 individuals were confirmed infected (Centers for Disease Control and Prevention 2010).

Globally, the disease currently is estimated to cause 220,000 to 600,000 deaths and 16 to 22 million illnesses per year, predominantly in children of school-age or younger in the developing world (Ivanoff, Levine et al. 1994; Crump, Luby et al. 2004; Ochiai, Acosta et al. 2008). Typhoid fever is one of the most common etiological sources of bacteraemia in many developing countries, with most of the cases being originated in the Indian subcontinent of South Asia (Bhutta 1996; Crump, Luby et al. 2004). Within the countries, the disease burden, however, is often heterogeneously distributed with the areas with poor hygiene and sanitation

suffering the most (Figure 1-1). A population-based study in India reported that the incidence of typhoid fever in the urban slums of Delhi was 9.8 per 1,000 persons per year (Sinha, Sazawal et al. 1999). The incidence was found to be three times higher in children below the age of 5 years compared to other age groups. Another study in Bangladesh estimated the incidence in urban slums to be 19 per 1,000 persons per year in preschool-aged children (Brooks, Hossain et al. 2005; Naheed, Ram et al. 2010). A retrospective analysis of *Salmonella* infection data from an urban hospital in Kathmandu, Nepal, assessed the frequency of *Salmonella* isolation from outpatients and inpatients over the period of 1993-2003 (Maskey, Basnyat et al. 2008). Among 82,467 blood cultures performed, *Salmonella* accounted for 75% (9,124) of all growths where 71% were *S*. Typhi and 29% were *Salmonella enterica* serotype *serovar* Paratyphi A (*S.* Paratyphi A). When comparing the period of 1997-2000 to the period of 2001-2003 for the proportion of total blood cultures that were taken, *Salmonella* septicaemia rates significantly increased from 6% to 14%. High disease burden from this region is also evident from the fact that most reported and identified cases in developed countries have had travel history to the region (Lynch, Blanton et al. 2009; Clark, Daneshvar et al. 2010).

Figure 1-1: Estimated global disease burden of typhoid fever and provincial disease burden in Vietnam

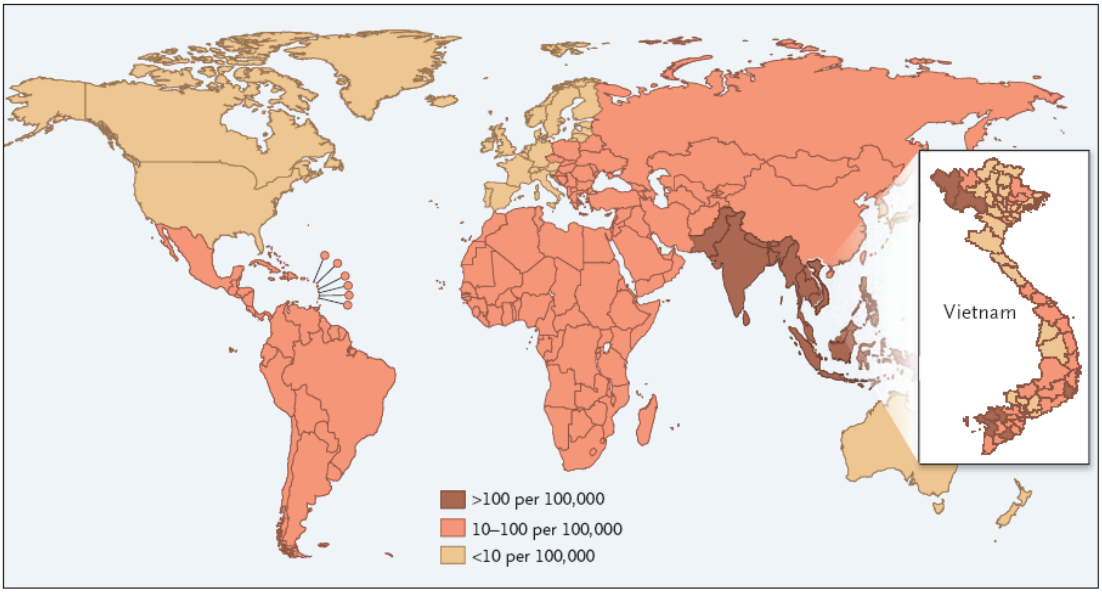


Figure taken from DeRoeck et al. (DeRoeck, Jodar et al. 2007) – The map showing country-specific incidence rates for the year 2000 from Crump et al. (Crump, Luby et al. 2004) and provincial incidence rates from Vietnam from a meta-analysis conducted by the DOMI Programme.

Several studies were carried out in typhoid endemic regions in Asia to determine potential risk factors. Risk factors relating to the waterborne characteristics of the disease were: consumption of water at the work site (Luby, Faizan et al. 1998); consumption of non-boiled untreated spring water (Mermin, Villar et al. 1999; Swaddiwudhipong and Kanlayanaphotporn 2001); consumption of water from non-municipal water source (Gasem, Dolmans et al. 2001); and consumption of contaminated tap water (King, Chen et al. 1989; Khan, Ochiai et al. 2011). Risk factors relating to the food borne characteristics of the disease were: consumption of ice cream (Luby, Faizan et al. 1998); and consumption of food from outdoor vendors (Velema, van Wijnen et al. 1997; Luby, Faizan et al. 1998; Vollaard, Ali et al. 2004). Other independent risk factors were: higher population density (King, Chen et al. 1989; Khan, Ochiai et al. 2011); unsanitary living conditions (King, Chen et al. 1989); poor hygiene and hygienic practices (Velema, van Wijnen et al. 1997; Gasem, Dolmans et al. 2001); low socio-economic level (Luxemburger, Chau et al. 2001); and recent contact to a typhoid fever patient (Luxemburger, Chau et al. 2001). Even though these studies highlighted the commonly known risk factors for *S.* Typhi infection, it is important to note that these studies employed different designs to recruit the cases and controls. Regarding cases, some of the cases were identified through community-based surveillance, while some others were through hospital-based surveillance. For the controls, similarly, some were matched community controls and some were matched hospital controls with or without fever. As controls should be from a reference population of the cases, non-standardized selection criteria for control subjects will be prone to bias in determining the common risk factors. Thus such differences in study design may have influenced the degrees of association and the outcomes in some of the above-mentioned studies.

1.2 Organism

The disease which we now call typhoid fever has been documented from ancient time. Romans and Greeks, including Hippocrates, had documented acute febrile illnesses that were often fatal (Cunha 2004). The disease was initially believed to have been caused by “typhos” – Greek word for smoke or pungent odour – which may be related to the smell from open

sewage. Because of the similar symptoms from other febrile illnesses, typhoid fever was often confused with murine typhus and malaria. The causative agent was identified in the late 19th Century. Browicz suggested that a Gram-negative bacillus was the cause of typhoid fever in 1874 and Klebs also described a Gram-negative organism associated with typhoid fever in 1880 (Browicz 1874; Klebs 1884). However, the identification of *Salmonella enterica* serovar serotype Typhi as the causative agent of typhoid fever is usually credited to Karl Eberth in 1880 (Eberth 1880). By 1884, Gaffky was able to isolate the bacteria by culture, and in 1885, Salmon and Smith described a serotype that is now known as *S.* Choleracsuis, the first bacteria that was known at that time to affect both human and animals (Gaffky 1884; Salmon and Smith 1886). Knowing the causative agent has also led to differentiation of typhus and typhoid fever. *Salmonella enterica* serovar serotype Typhi (*S.* Typhi), belongs to the *Salmonella* family, while typhus is caused by *Rickettsia*.

Salmonella belongs to the group of enterobacteriacae that are aerobic and Gram-negative (Parry, Hien et al. 2002; Bhan, Bahl et al. 2005). Currently there are approximately 2,400 pathogenic species of *Salmonella*. *Salmonella* possesses a flagellar antigen (H), somatic (O) and a surface antigen Vi. *Salmonella* are divided into two subspecies *S*. enterica and *S*. bongori. *S*. bongori contains eight serovars while *S*. enterica contains the other serovars that are divided into six subspecies based on flagellar H antigen. The antigenic formulae of *Salmonella* serotypes are defined and maintained by the WHO Collaborating Centre for Reference and Research and at Research for *Salmonella* at the Pasteur Institute, France. Currently, serotypes of *Salmonella* are differentiated using the technique recommended in the Kauffman-White scheme (Brenner, Villar et al. 2000).

S. Typhi is serologically placed in *Salmonella* group D due to O antigen 9 and 12. The structure of the organism is relatively stable with a few exceptions of isolates from Indonesia that have slightly different flagellar antigens where a unique flagella type, Hj, is present (also known as *Salmonella* Paratyphi Java). *S.* Typhi is highly stable serologically compared to other *Salmonella* serotypes (Mehta and Arya 2002). *S*. Typhi expresses a polysaccharide capsule Vi (virulence antigen) on its surface. Presence of Vi prevents the binding of O antigen to the O antibody and thus enables the pathogenesis of the organism. Humans are the only

restricted host for *S*. Typhi. The Vi capsular antigen is largely restricted to *S.* Typhi, although it is shared by some strains of *S. enterica* Group C and *Citrobacter freundii.* Clinical severity of typhoid fever is a result of the Vi antigen that increases the infectivity (Robbins and Robbins 1984). Vi expression is inhibited *in vitro* during repeated freezing and thawing, and in some cases, after several generation of growth. However, Vi negative strains have also been identified near-*in vivo* (immediate samples for blood culture) therefore Vi presence is not necessary for *S.* Typhi to cause typhoid fever illness (Baker, Sarwar et al. 2005; Wain, House et al. 2005). However the number of clinical cases of typhoid fever illness with *S.* Typhi lacking the *via*B locus gene – an operon that contains two genes that encode enzymes for the biogenesis and translocation of Vi – is very limited, hence, the clinical association of Vi negative strains need further investigation (Hornick, Greisman et al. 1970; Hone, Attridge et al. 1988; Baker, Sarwar et al. 2005). In vitro studies have shown that Vi antigen of *S*. Typhi has anti-opsonic and antiphagocytic characteristics, that reduces the level of secretion of *S.* Typhi-induced tumour necrosis factor alpha by human macrophages, and increases the level of resistance of the organism to oxidative killing (Wong, Feeley et al. 1974).

Genotyping of the bacterium was completed in 2000 (Parkhill, Dougan et al. 2001; Roumagnac, Weill et al. 2006; Baker, Pickard et al. 2008). Genetically, *S.* Typhi is monophyletic and diversity in sequences is limited (Kidgell, Reichard et al. 2002). However, the use of multi-locus sequence typing (MLST) or single nucleotide polymorphism (SNP) typing allowed accurate differentiation and developing the phylogenetic tree of the *S.* Typhi isolates from different parts of the world (Maiden, Bygraves et al. 1998; Achtman 2008). This method potentially enables tracking of endemic *S.* Typhi in a specific setting. Several genetically distinct *S.* Typhi haplotypes were identified circulating in an urban area of Jakarta, Indonesia, by using SNP typing (Baker, Holt et al. 2008). Similar studies were also conducted in Kenya and Nepal showing the haplotypic diversity of S. Typhi (Holt, Baker et al. 2010; Kariuki, Revathi et al. 2010). Another study detected few examples of unique forms of genetic variations such as insertions and deletions through SNP typing, which could potentially be used as markers for studying *S.* Typhi populations (Holt, Parkhill et al. 2008).

1.3 Pathogenesis

Susceptible human hosts usually ingest *S.* Typhi through contaminated food or water. The first barrier to food borne or waterborne diseases is the gastric acid in the stomach. Thus the inoculum and the type of vehicle in which *S.* Typhi is ingested influence the attack rate and the incubation period of the infection. A series of challenge studies in the 1960s have shown doses of 10^9 and 10^8 pathogenic *S.* Typhi ingested by volunteers in 45 ml of skim milk induced clinical illness in 98% and 89% of study volunteers, respectively; doses of 10^5 organisms caused typhoid fever in 28 to 55% of volunteers; and none of the 14 volunteers who had received 10^3 organisms developed clinical illness (Hornick, Greisman et al. 1970).

Inside the small intestine, *S.* Typhi attach to intestinal cells. They penetrate the mucosal epithelium to reach lamina propria through enterocytes and M cells, the domelike epithelial cells that cover Peyer's patches (Kohbata, Yokoyama et al. 1986). In the lamina propria, *S.* Typhi elicits an influx of macrophages and dendritic cells that ingest the organisms but do not generally kill them. Some remain within macrophages of the small intestinal lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes where there is further multiplication and ingestion by the macrophages. Eventually, there is a release of tumour necrosis factor-alpha, interleukin-2 (IL-2), IL-6, and other inflammatory cytokines by the mononuclear cells. After reaching the blood circulation via the thoracic duct, the bacteria are filtered from the circulation and sequestered inside the phagocytic cells of the liver, spleen, and bone marrow.

This “primary bacteraemia” allows *S.* Typhi to reside for relatively long period in the organs before inducing clinical systemic illness. Incubation period is usually 8-14 days. During the primary bacteraemia that follows ingestion of typhoid bacilli and seeding of the reticuloendothelial system, organisms also reach the gallbladder, an organ for which *S.* Typhi has a remarkable predilection (Gilman, Islam et al. 1979). Clinical illness is accompanied by a low level “secondary” bacteraemia which could also cause re-infection of the Peyer’s patches due to excretion of bacteria in bile.

S. Typhi can be readily cultured from bile or from bile-stained duodenal fluid in patients with acute typhoid fever (Hoffman, Punjabi et al. 1984; Avendano, Herrera et al. 1986). In approximately 2-5% of patients, the gallbladder infection becomes chronic (Ames and Robins 1943; Levine, Black et al. 1982). The tendency to become a chronic carrier is greater in females and increases with age at the time of acute *S.* Typhi infection, resembling the epidemiology of gallbladder disease (Ames and Robins 1943; Levine, Black et al. 1982). Carriers shed as many as 10^9 organisms/g faeces (Parry, Hien et al. 2002). *S.* Typhi remains in the gastrointestinal tract of the carriers without penetrating and without causing disease in the host.

1.4 Clinical picture

Clinical picture of typhoid fever is of a sub-acute systemic infection with diverse manifestations that can include effects on the gastrointestinal tract, nervous and cardiac system, and rarely a severe sepsis syndrome. The fever can be very high and patients can have a typical affect with somnolescence and apathy (Bhan, Bahl et al. 2005; Maskey, Day et al. 2006). Typhoid fever was recognized as a distinct disease in the earlier quarter of 19^{th} century and soon gained significance as a serious health problem due to its ability to spread quickly in populations, especially those living in congested areas such as urban settlements and military barracks. Although the disease is known widely, typhoid fever is still often confused with other acute febrile illnesses such as malaria, typhus and dengue fever, even with the use of laboratory diagnosis (Parry, Hoa et al. 1999; Ellis, Fukuda et al. 2006; Joshi, Rajeshwari et al. 2008).

Typhoid fever presents variably ranging clinical manifestations from mild fever to more severe forms such as toxic shock; however general symptoms include high grade fever (40^oC) lasting for more than 3 days, profuse sweating, altered bowel functions from constipation in adults to diarrhoea in children, malaise, myalgia, anorexia, and nausea (Levine 1999; Mweu and English 2008). Generally, typhoid fever progresses through four stages if proper treatment is not given timely (Levine 1999). In the first week, there is a slow rise in body temperature with

relative bradycardia, malaise, headache and cough. In some cases bleeding from nose and abdominal pain is also possible. The number of circulating white blood cells decreases with eosinopenia and relative lymphocytosis, blood culture become positive for *S.* Typhi, while Widal agglutination test is negative in the first week (Chatterjee, Jagdish et al. 2001; Mweu and English 2008). In the second week, fever reaches around 40^{o}C accompanied by slow pulse rate. Delirium is frequent and the patient is generally calm, but sometimes can become agitated. Rose spots appear on the lower chest and abdomen in around 30% of patients. Abdominal symptoms become more obvious with pain in the right lower quadrant. Diarrhoea with a frequency of 6 to 8 stools may occur during this time, however, constipation is also frequently seen. Spleen and liver become palpable and tender. Elevated transaminases can be seen on liver enzyme tests. Anti O and Anti H on Widal agglutination test become strongly positive, blood culture may also be positive depending on the quantity of blood taken from the patient. The third week of fever is characterized by the appearance of complications such as intestinal haemorrhage, encephalitis, metastatic abscess, cholecystitis, endocarditis and osteitis. Overall 10 to 15% of typhoid fever cases develop complications if not properly treated at early stage of the illness (Bhutta 1996). Intestinal perforation may occur in 1 to 3 % of cases and leads to peritonitis and ultimately to death if proper surgical intervention is not instituted urgently (Parry, Hien et al. 2002). In the fourth week, the fever is still high, with little oscillation. The patient may have delirium due to dehydration. Some of the rare complications include hepatic, splenic and bone marrow granulomas, splenic and liver abscesses, pleural effusion, phagocytic syndrome, pseudotumour cerebri, haemolytic endocarditis, and pericarditis. Arrhythmia or cardiogenic shock caused by toxic myocarditis with fatty infiltration of the heart had also been reported (Butler, Islam et al. 1991; Parry, Hien et al. 2002; Bhan, Bahl et al. 2005). Typhoid fever may also complicate pregnancy leading to miscarriage. Vertical transmission has been documented and may result in a neonatal typhoid (Reed and Klugman 1994; Mohanty, Gaind et al. 2009).

Hospitalization rates of typhoid fever cases vary from 10 to 40%, while the rest either self-medicate or are treated on an outpatient basis (Parry, Hien et al. 2002; Ochiai, Acosta et al. 2008). However population-based studies have reported variation in hospitalization rates. In settings where early treatment was provided due to extensive and systematic surveillance, it

has proved possible to treat typhoid early. On the other hand patients who had access to only the routine health system had higher rates of hospitalization and subsequent complications. The average length of hospital stay ranges from 10 to 15 days. Following recovery, convalescing patients may continue to excrete *S*. Typhi in the faeces for up to 6 months. One to 4% of patients become long-term carriers, excreting the organism for more than one year, who have an elevated risk of hepatobiliary cancers. Most carriers are asymptomatic. In the pre-antimicrobial era, typhoid was a debilitating disease with a mortality rate as high as 30% (Woodward, Smadel et al. 1948; Chatterjee, Jagdish et al. 2001). The case fatality rate is variable among the endemic countries, with Pakistan and Vietnam having a case fatality rate of less than 2 percent and Indonesia and Papua New Guinea as high as 30 to 50% (Hoffman, Punjabi et al. 1984; Punjabi, Hoffman et al. 1988; Bhutta 1996; Hoa, Diep et al. 1998). The case fatality rates are higher among children under one year of age and among elderly (Stuart and Pullen 1946; Butler, Islam et al. 1991). However, the most significant contributor to mortality is a delay in providing effective antibiotic treatment. Current estimates of case fatality of the disease range from one to four percent, including treated patients. Left untreated, case fatality can climb to 10-20%. Case fatality rates have been found to be ten times higher in children younger than 4 years compared to older children (Butler, Islam et al. 1991; Akpede and Akenzua 2001; Thaver, Zaidi et al. 2008).

1.5 Treatment (antimicrobials and antimicrobial resistance)

Basic supportive measures, such as oral or intravenous hydration, provision of antipyretics, and appropriate nutrition, are important in treating typhoid fever cases. However, patients presenting with serious complications, such as persistent vomiting, severe diarrhoea, and/or abdominal distension will require immediate hospitalization and appropriate antibiotic therapy.

Chloramphenicol was the standard antimicrobial for treating typhoid until the emergence of chloramphenicol-resistant strains (Herzog 1976; Butler, Rumans et al. 1982). Outbreaks of *S.* Typhi strains resistant to chloramphenicol first appeared in 1970s in several parts of the world. As new drugs such as ampicillin and co-trimoxozole became available, resistance against

these antimicrobials also emerged. Outbreaks of multi-drug resistance (MDR) defined as resistance to first-line antibiotics (chloramphenicol, amoxicillin or trimethoprim-sulfamethoxazole) was first reported in late 1980s in South Asia and the Middle East that later spread to East Asia and Africa (Rowe, Ward et al. 1997; Rahman, Siddique et al. 2006; Hasan, Zafar et al. 2008). In southern Vietnam, over 70% of isolates were found to be multi-drug resistant in 1990s (Hoa, Diep et al. 1998). MDR typhoid has been associated with more severe illness and higher rates of complications and deaths, especially in children under two years of age (Bhutta 1996). The emergence of MDR *S.* Typhi strains has also led to the wide-spread use of fluoroquinolones, such as ciprofloxacin and ofloxacin, in countries where MDR is a problem. However, outbreaks of nalidixic acid resistant typhoid started to occur in Vietnam and Tajikistan in early 1990s and then spread to Pakistan and India (Wain, Hoa et al. 1997; Mermin, Villar et al. 1999; Mandal, Mandal et al. 2004; Chau, Campbell et al. 2007). Nalidixic acid resistant typhoid cases does not respond well to fluoroquinolone treatment, exhibiting more prolonged fever than sensitive cases, and, in one study, a ten-fold higher rate of post-treatment stool carriage was observed in resistant cases as compared to sensitive cases (20% vs. 1.8%), increasing their potential to infect others. Cases of full-blown resistance to ciprofloxacin are also reported from Pakistan, India, Tajikistan, Nepal, and other areas (Bhutta, Naqvi et al. 1991; Gupta, Kumar et al. 1993; Rowe, Ward et al. 1995; Murdoch, Banatvaia et al. 1998; Chitnis, Chitnis et al. 1999; Threlfall, Ward et al. 1999; Kato, Fukayama et al. 2007; Maskey, Basnyat et al. 2008).

Currently the fluoroquinolones are considered as the optimal choice for the treatment of typhoid fever in adults and also in children. However, the emergence of fluoroquinolone resistance and reduced treatment outcomes with fluoroquinolones due to nalidixic acid resistance suggest that the use of fluoroquinolones in primary care settings must be observed carefully. In areas where the fluoroquinolones are not available or not registered for public health use and where *S.* Typhi is still sensitive to traditional first-line antibiotics, these remain appropriate for the treatment of typhoid fever (World Health Organization 2003). Azithromycin, though relatively more expensive, has shown very good clinical results in the treatment of MDR and nalidixic acid resistant typhoid fever (Chinh, Parry et al. 2000; Parry, Ho et al. 2007). More recently, a trial was conducted to compare the efficacy of gatifloxacin to azithromycin in

Vietnam. The treatment course with gatifloxacin, a newer fluoroquinolone, was shown to be not inferior to that with azithromycin in the trial. Gatifloxacin, thus, is another recommended antimicrobial for the treatment of MDR or nalidixic acid resistant typhoid fever, considering the lower cost – only approximately one-third of the cost of azithromycin (Dolecek, Tran et al. 2008).

1.6 Prevention and vaccines

Improvement in sanitary infrastructures and implementation of hygienic practices can reduce the disease burden as seen in most developed countries. However, the development of an adequate infrastructure for improved water and sanitation requires large investments, and is therefore a far-off target for the impoverished populations in the developing world. Basic health education such as hand washing and food handling is also known to be effective in reducing typhoid fever. Furthermore, there are licensed vaccines to prevent typhoid fever. Although typhoid fever can be effectively treated with antibiotics, growing rates of antibiotic resistance in many countries are making this treatment option increasingly more difficult and costly. Given this fact, it seems essential to consider a comprehensive approach that combines targeted vaccination of high-risk populations as a short- to medium-term measure, combined with the longer term solutions of water and sanitation improvements and elevated living standards, while ensuring appropriate treatment procedures at every level of the health care system.

Vaccines against typhoid fever are not new. Inactivated phenol-preserved whole-cell typhoid vaccines have been available since the 1890s. Although production was poorly standardized, the vaccine showed a considerable reduction in typhoid fever incidence in British and United States soldiers where it was used at large scale (Wright and Leishman 1900; Hawley and Simmons 1934; Siler and Dunham 1939). During 1960s and 1970s, randomized controlled field trials of this inactivated parenteral whole cell vaccine were sponsored by the WHO in Eastern Europe and Guyana. The vaccine was moderately efficacious (51-88%) in children and young adults in preventing typhoid fever, and the protection persisted for up to 7 years

(Ashcroft, Ritchie et al. 1964; Hejfec, Salmin et al. 1966; Ashcroft, Singh et al. 1967; Tapa and Cvjetanovic 1975). However, their high levels of reactogenicity; fever (up to 30% of the vaccinees), headache (up to 10%), and severe local pain (up to 35%), led to the removal from public health programs in most countries (Wahdan, Sippel et al. 1975; Ivanoff, Levine et al. 1994; Engels and Lau 2000). Two new-generation vaccines have been available in the market since late 1980s to early 1990s: the oral live attenuated Ty21a and injectable Vi polysaccharide vaccine (Table 1-1).

The Ty21a vaccine consists of a mutant strain of *S.* Typhi Ty2, which was isolated after chemical mutagenesis and has a *gal*E- and Vi-negative phenotype (Germanier and Fuer 1975). Immune response to the vaccine starts 14 days after vaccination, which is mediated by mucosal (IgA), serum (IgG), and cell-mediated antibodies. The overall protective efficacy for a three-dose regimen ranged between 67% and 80% in large-scale efficacy trials, conducted in 1980s in Chile (Wahdan, Serie et al. 1982; Levine, Ferreccio et al. 1990). Two different formulations were tested in typhoid-endemic regions of Indonesia in a randomised, double-blind trial design (Simanjuntak, Paleologo et al. 1991). More than 20,000 subjects aged 3 to 44 years received either three doses of enteric coated capsules containing placebo or live Ty21a, or three doses of lyophilised placebo or live Ty21a reconstituted with phosphate buffer. During 30 months of follow-up, the incidence rates of culture-confirmed typhoid fever were 379/100,000 per year for subjects who received the liquid formulation of vaccine and 468/100,000 per year for subjects who received enteric coated capsules; and among controls the incidence rate was 810/100,000 per year. The protective efficacies of the liquid and enteric coated formulations were 53% and 42%, respectively (Table 1-2). The most common adverse events reported with Ty21a were mild and transient gastrointestinal disturbances, followed by general symptoms such as fever (Engels and Lau 2000). Only a limited and transient level of shedding in the stools of volunteers was seen with Ty21a and no person-to-person transmission was observed (Wahdan, Serie et al. 1982; Levine, Tacket et al. 2001). Further, the mutations of Ty21a have been genetically stable. Reversion to virulence has not been observed in vitro or in vivo (Gilman, Hornick et al. 1977). This vaccine is currently produced by Berna Biotech (a Crucell company) in Switzerland and Boryung in South Korea

and licensed for use in persons two years and above for the liquid formulation and five years and older for the capsule formulation.

During the same period, the US National Institute of Health (NIH) purified the capsular polysaccharide, Vi, for its use as a vaccine antigen. This subunit vaccine was developed from wild type S. Typhi strain Ty2 on the basis of non-denatured purification of the Vi polysaccharide. Vi consists of ([alpha]1-4),2-deoxy-2-N-acetyl galacturonic acid, which is partially O-acetylated at carbon 3 and forms a capsule that protects the bacteria against complement-mediated lysis and phagocytosis (Robbins and Robbins 1984; Looney and Steigbigel 1986). Vi elicits anti-Vi serum IgG antibodies in 85% to 95% of adults or children older than 2 years of age (Tacket, Ferreccio et al. 1986). The protection conferred by and immunological response to the vaccine were consistent both in populations of endemic, as well as non-endemic areas (Acharya, Lowe et al. 1987; Klugman, Gilbertson et al. 1987; Mirza, Wamola et al. 1995; Klugman, Koornhof et al. 1996). The injectable Vi polysaccharide vaccine is given in a single-dose and was found to confer, overall, 64-72% protection for 17-21 months and 55% over three years (Table 1-3). Vi polysaccharide vaccine is well tolerated and safe. The most common side-effects are pain, redness and induration at injection site, and fever. In very rare cases, allergic reactions and rashes have been observed (Hessel, Debois et al. 1999). Like other polysaccharide vaccines, Vi is not immunogenic in children less than two years of age, and thus is licensed for use in persons two years and above. As there was no patent protection to the production technology of the Vi polysaccharide vaccine, developing country manufacturers also began producing the same vaccine since early 1990s. Currently there are at least 3 manufacturers exporting the vaccine (sanofipastuer, GlaxoSmithKline Biologicals, and Bharat Biotech [India]) and many other companies producing for local use (e.g. Lanzhou Institute [China], Chengdu Institute [China], Finlay Institute [Cuba], DAVAC [Vietnam]).

Table 1-1: Characteristics of the two new-generation typhoid fever vaccines; Ty21a and Vi polysaccharide

Characteristic	Live attenuated Ty21a	Vi capsular polysaccharide
Vaccine type	Live attenuated	Subunit
Composition	Chemically-mutated Ty2 strain of S. Typhi	Purified Vi capsular polysaccharide of the Ty2 S. Typhi strain
Immunogenic properties	▪ Elicits mucosal IgA and serum IgG antibodies against O, H and other antigens, as well as cell-mediated responses ▪ No booster effect has been shown	▪ Elicits serum IgG Vi antibodies ▪ T-cell independent (no booster response)
Route of administration	Oral	Parenteral (subcutaneous or intramuscular)
Minimum age vaccine is licensed for use	Two years old for liquid formulation and five years old for capsule formulation	Two years old
Characteristic	Live attenuated Ty21a	Vi capsular polysaccharide
Formulation	▪ Enteric-coated capsules, or ▪ Liquid suspension (lyophilized vaccine + buffer mixed with water upon use)	Solution of 25 µg combined with buffer
Number of doses required for complete vaccine regimen	Three to four	One
Storage requirements	Requires storage at 2º to 8ºC	Requires storage at 2º to 8ºC
Shelf life in higher temperature	14 days at 25 °C	6 months at 37 °C 2 years at 22 °C
Safety/tolerability	High	High
Efficacy at 3 years (95% CI)	51% (36%, 62%)	55% (30%, 70%)
Length of protection	At least 5-7 years	At least 3 years

Table 1-2: Summary of studies undertaken for the Ty21a vaccine in developing countries

Study (Year)	Formulation	No. Study Subjects	Ages (Years)	Follow-up Period	PE for Blood Culture Confirmed Typhoid (95% CIs)	Incidence Rate in Control Group (per 100,000)
Alexandria, Egypt (1978-80)	Liquid given with tablet of $NaHCO_3$	32,388	6-7	36 months	96% (77-99%)	50
Area Occidente, Santiago, Chile (1983-86)	3 doses of enteric-coated capsules given (1-2 days between doses)	140,000	6-19	36 months 7 years	67% (47-79%) 62%	110
Area Sur Oriente, Santiago, Chile (1986)	3 doses of enteric-coated capsules (1-2 days between doses)	81,321	6-19	3 years	33% (0-57%)	100
	3 doses liquid suspension (1-2 days between doses)			3 years 5 years	77% 78%	
Sumatra, Indonesia (1986-1989)	3 doses of enteric-coated capsules (7 days between doses)	20,543	3-44	30 months	42% (23-57%)	810
	3 doses liquid suspension (7 days between doses)				53% (36-66%)	

Table 1-3: Summary of studies undertaken for the Vi polysaccharide vaccine in developing countries

Study (Year)	Formulation	No. Study subjects	Ages (Years)	Follow-up period	PE for Blood culture confirmed typhoid (95% CIs)	Incidence rate in control group (per 100,000)
Kathmandu Valley, Nepal (1986-1988)	1 dose of Vi (25 µg)	6,907	5-44	17 months	72% (42-86%)	926
E. Transvaal, South Africa (1985-1988)	1 dose of Vi (25 µg)	11,384	6-14	21 months 36 months	64% (36-79%) 55%	773
Quan County, Guangxi Province, China (1995-1997)	1 dose of locally-produced Vi (30 µg)	131,271	3-50 (92% school-age)	19 months	69% (28-87%) (72% in school children)	63-78

The development of these new vaccines, together with the emergence of antibiotic-resistant *S.* Typhi strains and the continued high burden of the disease, led the WHO in 1999 to recommend immunization using the new-generation typhoid vaccines in school-aged children and young adults *"in areas where typhoid fever in these age groups is a significant problem and particularly where antibiotic resistant* S. Typhi *strains are prevalent… until socio-economic improvements finally interrupt transmission of* S. Typhi*"* (World Health Organization 2000).

Unfortunately, the use of new-generation typhoid vaccines by the public sector has been limited to only three countries and only on a limited basis (DeRoeck, Ochiai et al. 2008). Several provinces and districts in China began mass vaccination campaigns for school children and food handlers in mid-1990s, using locally-produced Vi after the US NIH transferred the technology to six state-run vaccine producers in the country. These programs have reportedly been effective in dramatically reducing typhoid incidence in some areas in Southwest China (e.g. parts of Guangxi Zhuang Autonomous Region). The Vietnam National Immunization Programme also began annual campaigns in 1997, using at first imported and later locally-produced Vi for 3-10 year old children in a limited number of high-risk districts. However, Vietnam's programme is considered too limited to effectively control the disease throughout the country. And finally in 2004, Delhi State, India introduced Vi vaccination in 2-5 year olds through community-based campaigns.

1.7 Diseases of the Most Impoverished Programme

Attention to diseases which predominantly affect developing countries was limited at the close of the 20th century. Typhoid fever was one of them. Vaccine manufacturers did not consider vaccines against these diseases as profit-making and there were significantly less efforts in research and development especially from the private sectors. The needs were thus clear in international public health arena to put significant money and effort in supporting implementation research to accelerate the development and introduction of vaccines against those diseases. One of the pioneers was the Diseases of the Most Impoverished (DOMI)

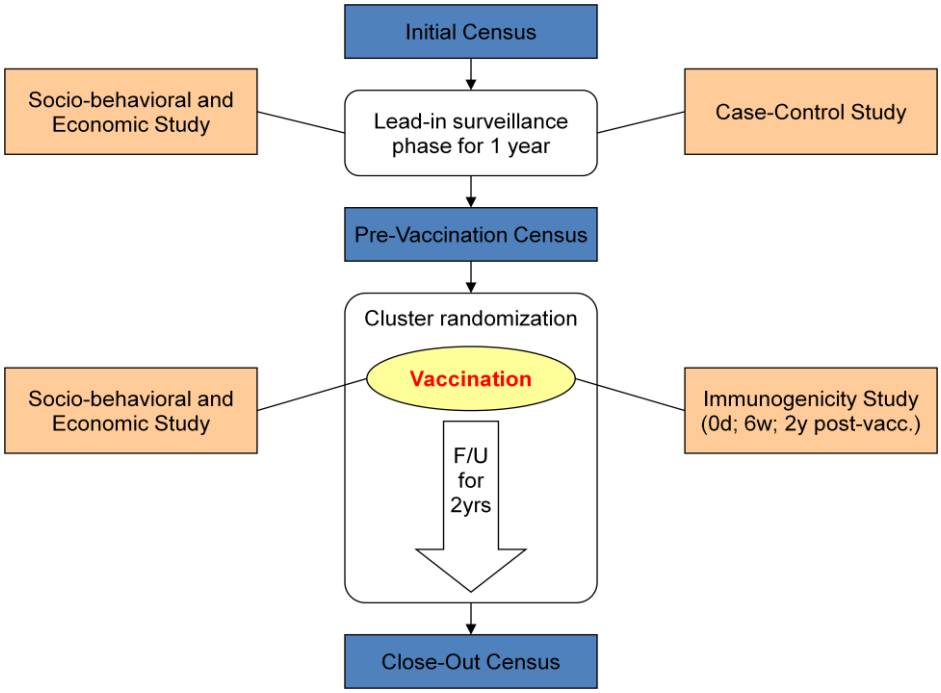
Programme, funded by the Bill and Melinda Gates Foundation and implemented by the International Vaccine Institute. The DOMI Programme focused on *Shigella*, Cholera, and Typhoid. The DOMI Programme was needed because at the time of inception, there were very limited data available on these three diseases to push the introduction agenda forward.

The lack of uptake of the typhoid vaccination in endemic countries was discussed during policymaker surveys in Asia conducted in 2000-2001 as part of the DOMI programme (DeRoeck, Clemens et al. 2005). Many developing countries are uncertain of their true typhoid disease burden, due to the lack of inexpensive rapid diagnostic tools, infrequency of laboratory testing, poor disease reporting systems and the fact that the clinical diagnosis of the disease is often confused with other febrile illnesses. In some countries, there may also have been political pressure for local government officials not to report typhoid cases, since they are considered an indicator of inadequate or failing water and sanitation systems. There has also been a sense of complacency created by the introduction of relatively inexpensive antibiotics several decades ago, which were effective in substantially reducing typhoid-related deaths, but which have progressively become ineffective due to the acquisition of antibiotic resistance by the bacterium. In addition, with some exceptions, typhoid tends not to cause major epidemics that command the public's attention, but extracts its toll of morbidity and mortality as an endemic illness. Other barriers to greater public sector use of these vaccines include: the relatively high prices charged by international producers of the vaccines on the world market; a preference among policymakers for water and sanitation improvements over vaccination to control enteric diseases; a lack of awareness among many policymakers about the new-generation vaccines; and uncertainty of the logistic feasibility of mass vaccination of children outside of the infant EPI schedule in their countries. Furthermore, the peak age group for typhoid in many settings is from 5 to 15 years, which falls outside of the under-five year age group that has been the focus of most international public health efforts on vaccines.

The design of the DOMI programme allowed us to address many of the uncertainties prevalent among policymakers. Multi-centric multi-disciplinary studies of the typhoid programme were designed to fill the gaps towards public health introduction of the vaccine (Ochiai, Acosta et al. 2007). The pillar of the programme was multi-centric disease

surveillance platforms in five countries, which were complemented with economic studies to estimate the cost of typhoid fever illness from both public and private practices, socio-behavioural studies to understand the population demand and acceptance to the vaccination, epidemiologic studies to measure the incidence in standardized method, effectiveness trials to measure the impact of the vaccine and its acceptance, and integration of all to compute the cost-effectiveness of the vaccination (Figure 1-2).

Figure 1-2: DOMI Programme's Multi-centric multi-disciplinary study design



The programme concentrated on six Asian countries with endemic typhoid fever. Several considerations led DOMI to focus on Vi polysaccharide vaccine rather than Ty21a vaccine as a tool for accelerated introduction (Table 1-4). First, Vi polysaccharide vaccine is given as a single dose, as opposed to 3-4 strictly timed doses needed for Ty21a vaccine, making Vi polysaccharide vaccine better suited for developing country programs. Second, Vi polysaccharide vaccine is sold at prices as low as USD 0.50 and is produced by two international and several developing country vaccine manufacturers, whereas Ty21a vaccine is a more expensive vaccine and is produced only by a single international manufacturer. Third, two major developing countries in Asia, China and Vietnam, had already made a decision to introduce Vi polysaccharide vaccine, and both countries had established local production of this vaccine, made possible by the simple production technology for Vi polysaccharide vaccine and its non-patented status. In contrast, Ty21a vaccine is not used as a public health tool in any developing country, and its more complex production technology makes local production in developing countries difficult.

Table 1-4: Comparative characteristics of Vi polysaccharide and Ty21a vaccines

	Vi	Ty21a
Clinical properties		
Safety	High	High
Safety in HIV +	Safe	Must exclude HIV (+)
Efficacy	Moderate (not efficacious under 2 years of age)	Moderate (not efficacious under 2 years of age)
Protection inversely related to disease incidence	No	Yes
Duration of protection	At least three years	At least five years
Herd immunity	Likely but no data	Yes
Programmatic properties		
Number of doses	One	3-4 on alternate days
Cold chain requirements	Not strict	Strict
Supply, access and financing		
Licensed internationally	Yes	Yes
Technology transfer to emerging producers	Successfully accomplished by several emerging producers	Never accomplished

Interviews of Asian policymakers by the DOMI Programme revealed that uncertainties about the programmatic feasibility and impact under realistic conditions constituted important roadblocks for the use of Vi polysaccharide vaccine as a routine public health tool (DeRoeck, Clemens et al. 2005). As well, there were doubts about whether Vi polysaccharide vaccine was protective in children aged 2-5 years, and whether Vi polysaccharide vaccine was able to confer herd protection. To address these uncertainties, large-scale, cluster-randomized trials were conducted in the urban slums of Kolkata, India and Karachi, Pakistan, and two cities in China and Vietnam where typhoid fever is a major public health problem. While sites in Kolkata and Karachi included the pre-school age group (2 to 5 years), the other two sites carried out the trials only from age 5 and above. This was because the incidence of typhoid in younger age group was limited in these sites, and their current vaccination programs focus on school going children. In order to analyze the protection in the younger age group, the analyses for this thesis focus on data from Kolkata and Karachi sites.

1.8 Vaccine effectiveness trials

In order to scientifically estimate the impact of vaccine used under public health conditions, such as in DOMI Programme, cluster randomized design was proposed. A cluster randomized trial considers the social units (i.e. collection of individuals) rather than individuals. Though statistically cluster randomization is less efficient than individual randomization, there are certain benefits from employing this method (Cornfield 1978; Donner and Klar 2000). With the variation in real-life settings arising from the fact that infectious diseases such as typhoid fever tend to spread more efficiently and rapidly in a specific social context (e.g. family, school, etc.) and the possible effect of more frequent and relevant personal interactions among group members receiving the same intervention, cluster randomized design was considered to be more applicable to observe public health impact of the vaccine (Smith and Morrow 1996; Koepsell 1998). Especially for the latter point in the perspective of vaccine trial, individual randomization is not optimal because the herd protection may artificially provide protection to those who did not receive the intervention.

To understand the trial design, it is essential to differentiate between efficacy and effectiveness. While efficacy measures the performance of a vaccine in idealized settings, effectiveness is a term used to present the performance of a vaccine in real-life settings. This differentiation may not be clear from the perspective of the vaccine, but it has a clear difference when it comes to the population. An idealized setting is portrayed in a typical Phase III trial for vaccine licensure through individually randomized controlled design. This is to ensure the influence of other factors to be minimal to evaluate the vaccine's performance. This trial design will measure the direct reduction of host susceptibility to infection conferred by vaccination. Additionally, this design increases the statistical efficiency by requiring a minimum number of subjects. More important to note is the limited applicability of the trial results in individually randomized controlled design to real-life settings. Study participants are usually recruited in very stringent inclusion criteria and often requested to follow other restrictions, such as refraining from receiving other vaccines or taking certain medications. Efficacy trials are limited by their inability to predict public health impact of the vaccine use. Testing a vaccine in such real-life setting where there are limited restrictions for subject recruitment and subject activities can observe the effectiveness of the vaccine in community. Public health impact considers the vaccine's performance beyond the vaccinees, notably the herd protection or indirect effect, which can only be seen when vaccines are administered to groups rather than to individuals (Halloran, Haber et al. 1991; Clemens, Brenner et al. 1996; Halloran, Struchiner et al. 1997). The cluster randomized control design uses clusters as unit of randomization and allows to estimate not only the total protection, but also indirect and overall protections, which are very important measures for public health (Halloran, Struchiner et al. 1997).

Total protection is estimated by comparing the incidence of the outcome among vaccine recipients and the incidence of the outcome among control recipients. It reflects the combination of direct protection of vaccinees by the vaccine together with herd protection of vaccines on individuals not vaccinated within the vaccine clusters. Indirect protection is estimated by comparing the incidence of the outcome among non-recipients in the vaccine clusters and the incidence of the outcome among non-recipients in the control clusters. It reflects the herd protection of non-vaccinees in vaccine clusters. This provides the most

unbiased estimate of the indirect protection. Overall protection is estimated by comparing the incidence of the outcome among population in the vaccine clusters and the incidence of the outcome among population in the control clusters. It reflects the protection of the general population regardless of vaccine uptake.

2 AIM AND OBJECTIVES OF THE THESIS

2.1 Overall aim

To consider the applicability of typhoid vaccination as a mean to control typhoid fever by evaluating the effectiveness and immunogenicity of the Vi polysaccharide typhoid vaccine in urban slum areas of Kolkata, India, and Karachi, Pakistan, using cluster randomized controlled trial design.

2.2 Specific objectives

1. To establish population-based surveillance system to capture febrile cases from the study sites in Kolkata, India and Karachi, Pakistan;
2. To assess the typhoid disease burden from the communities using blood culture as endpoint;
3. To determine the effectiveness of the Vi polysaccharide typhoid vaccine in urban slum areas of Kolkata, India, and Karachi, Pakistan using cluster randomized controlled trial design;
4. To evaluate the immunogenicity of the Vi polysaccharide typhoid vaccine recipients in urban slum areas of Kolkata, India, and Karachi, Pakistan using Vi IgG ELISA; and
5. To consider the applicability of vaccination programme as a mean to control typhoid fever in areas similar to the study sites.

3 KOLKATA EFFECTIVENESS TRIAL

3.1 Introduction

Typhoid fever is considered to be endemic in most parts of India. Health surveys conducted by the Indian Federal Ministry of Health in the community development areas showed a morbidity rate from typhoid fever varying from 102 to 2,219 per 100,000 persons in different areas of the country. In a community-based longitudinal study using active surveillance for case detection in Delhi slum an overall incidence of 980/100,000 person-year was observed and children below 5 years of age had the highest incidence of 2,730/100,000 person-year (Sinha, Sazawal et al. 1999). There were no reliable contemporary data on typhoid fever epidemiology in North-western India. A health report by the government of West Bengal provided total reported cases of enteric fever from West Bengal to be 31,547 with 86 deaths in the year 1999, however, no information was provided on how and what basis the reporting was made.

In the late 1980s, *S.* Typhi resistant to standard antimicrobial agents was reported with alarming frequency from different parts of India, including Kolkata (Talawadekar, Vadher et al. 1989; Anand, Kataria et al. 1990; Ayyagari and Pal 1991). Small scale community studies and hospital-based studies have shown 50% of circulating strains were resistant to chloramphenicol and 65% to trimethoprim-sulphamethoxazole (Anand, Kataria et al. 1990). There were also reports of several treatment failures with ciprofloxacin (Dutta, Mitra et al. 2001).

The study was conducted in an urban slum site in Kolkata city, the capital of West Bengal, India. The Kolkata Municipal Corporation area has an estimated population of 6 million. There are 141 wards, each with a ward office responsible for public health activities and supervised by a medical officer. A contiguous area encompassing most of Ward 29 and all of Ward 30 in Eastern Kolkata, with a total population of about 60,000, was selected as the study site. The study population resided in legally registered urban slum areas with high population density.

Epidemiological data from the DOMI programme, as well as from other reports on typhoid fever indicated the area to be highly endemic of typhoid fever (Saha, Dutta et al. 2003; Sur, von Seidlein et al. 2006; Kanungo, Dutta et al. 2008; Ochiai, Acosta et al. 2008). The area also noted high incidence of antibiotics resistance (Anand, Kataria et al. 1990; Dutta, Mitra et al. 2001; Dutta, Sur et al. 2008). Chloramphenicol was the choice of antibiotics until large proportion of locally isolated *S.* Typhi was found to be resistant. However, more recently, there has been a report of rollback of chloramphenicol resistance since the use of chloramphenicol was discouraged (Dutta, Sur et al. 2005).

Because typhoid vaccine is a non-EPI vaccine in India and that the use of non-EPI vaccines is decentralized, the local health authorities had considered typhoid vaccination to be carried out in all age groups in a campaign fashion. As the data on incidence comes from all age groups, this was considered most beneficial to the community. The DOMI programme, therefore, planned the effectiveness trial in Kolkata considering this approach.

3.2 Overview of the study

The study was conducted collaboratively by the National Institute of Cholera and Enteric Diseases (NICED) in Kolkata and the International Vaccine Institute (IVI) in Seoul. 80 contiguous residential clusters of persons in the study area were randomized to receive either a single dose of Vi polysaccharide vaccine or a single dose of inactivated hepatitis A vaccine. Persons aged two years and older were eligible to receive vaccine. Vaccine protection against typhoid fever was monitored for two years after vaccination at both outpatient and inpatient facilities serving the study population. Vaccine safety was monitored by both active and passive surveillance. The primary outcome of the study, determined *a priori*, was total vaccine protection conferred by Vi polysaccharide vaccine over two years of follow-up, assessed by the comparative incidence rates of blood culture-proven typhoid fever in recipients of Vi versus hepatitis A vaccine.

3.3 Pre-study surveillance

The study was conducted in an urban slum site in Kolkata city, the capital of West Bengal, India. The Kolkata Municipal Corporation area has an estimated population of 6 million. There are 141 wards, each with a ward office responsible for public health activities and supervised by a medical officer. A contiguous area encompassing most of Ward 29 and all of Ward 30 in Eastern Kolkata was selected as the study site. These wards have high proportion of *bustee*, a legally recognized slum settlement, where provision of safe water and sanitary practices are considered to be more limited and at high risk area for typhoid fever. Reliable data on typhoid fever incidence in this population was unavailable. Nonetheless, it was widely believed by both clinical and public health personnel that the typhoid fever was widespread in this population. Households in the study area were densely grouped into premises. Each premise usually had a common latrine and water supply.

Studies conducted by the National Institute of Cholera and Enteric Diseases (NICED) have shown a literacy rate of 60%, major occupation of the people (65%) to be daily labour, and an average household income of approximately USD 50 per month in 2001. 64 percent of the population use drinking water from tube wells and 98% use latrines provided by the municipality.

In the Kolkata Municipal Corporation area as a whole, healthcare is provided by 7 referral government hospitals and various traditional practitioners, private clinics, and private nursing homes. Preparatory activities have been conducted in the study site. These included the census, an establishment of typhoid fever surveillance system, and the setting-up of 5 health outposts within the selected study wards (approximately one for every 10,000 population) and 2 at the referral hospitals selected: Infectious Diseases Hospital (IDH) and B.C. Roy Children's Hospital. The IDH, the referral centre for West Bengal, is in Beliaghata, about 3 kilometres from the study site. B.C. Roy Hospital, the referral centre for severely ill children, is adjacent to the study site.

Census

Two censuses were carried out to collect the household information at different time points. These censuses captured the *de jure* population. First census was carried out at the initiation of the preparation from January to March 2003 to identify and register residents with no plans to move within the next 3 years. Census teams visited each and every household in the study area and interviewed an adult member of the household. Though interviewing the head of the household was initially planned, it was not feasible as head of the household was usually out for work during the daytime when the census took place. Each household and each individual resident in the study area was assigned a unique study number and recorded in the project data management system. Second census was carried out from February to April 2004 to define the sampling frame for the vaccination, enumerating not only the demographic information of the residents from the first census, but also the new residents fulfilling the registration criteria (*vide infra*).

In wards 29 and 30 of Kolkata, which consists of legally recognized and registered urban slums (“*bustees”)* had a population of 56,946 in the initial study census conducted in 2003. The analysis of the first census showed 1,500 premises in the study area with an average of 7 households per premise. The household median number of years of residence was 35 years. Residents of the socio-economically better-off areas were excluded because of their known strong preference for private health care. Temporary residents of these areas were also excluded because of their increased likelihood of being lost to follow-up.

Surveillance

The disease surveillance system had been set up to prepare for the effectiveness trial. Age groups under surveillance were selected as those judged by local officials to be the most appropriate targets for typhoid vaccination: 2 years old and above (no upper age limit).

Five project health outposts were established (three in Ward 29 and two in Ward 30) and served as clinic and referral point for the patients from the area private clinics. Passive surveillance was augmented through community health workers visiting each household monthly to encourage febrile patients to visit participating healthcare facilities, and private practitioners were encouraged to refer patients for free typhoid and malaria diagnosis and

treatment at these outposts. Additionally, two outposts were set up in the nearby government hospitals (Infectious Diseases Hospital and B. C. Roy Children's hospital) since these two hospitals are frequented by the people from the study area. Augmented passive surveillance was considered necessary because there was no centralized system to report or treat typhoid fever patients in the area.

Patients residing in the catchment areas and presenting to a treatment facility, either the project health outpost or any of the private practitioners, with fever of 3 days or more were targeted to participate in the study. Surveillance included patients treated as outpatients as well as inpatients. Clinic activity for the enrolled cases consisted of systematic collection of clinical information, recorded on standardized forms, and collection of a single blood culture by venipuncture. Approximately 5 to 8 ml of blood was collected to inoculate Bactec Plus Aerobic® culture bottles (Becton Dickinson, New Jersey, USA); for younger children, about 3 to 5 ml of blood was inoculated into Bactec Peds Plus® culture bottles (Becton Dickinson, New Jersey, USA). Details on laboratory procedures are noted in section 3.4.7. Those who had less than 3 days of fever were given clinical assessment and encouraged to return to the outpost if the fever persists for more than 3 days.

An important feature of the surveillance was accurate identification of all patients attending the treatment facilities to determine the study participants. To ensure the identification, a computer-based identity search programme was developed using the project census data and equipped in all study health outposts. The computer-based identity search programme allowed active identity search while the patient was being attended by the physician. This identification process was conducted by the project health personnel from the community. Case report forms from private sectors without active identity confirmation were checked with the same programme at the data management unit retrospectively. Additionally, the culture positive cases were followed up to assess disability at days 7, 14, 30, and 60. At the time of the follow up on the day 7, study personnel were to confirm the identity of the individual so that no misclassification was to occur in the analyses.

Analysis

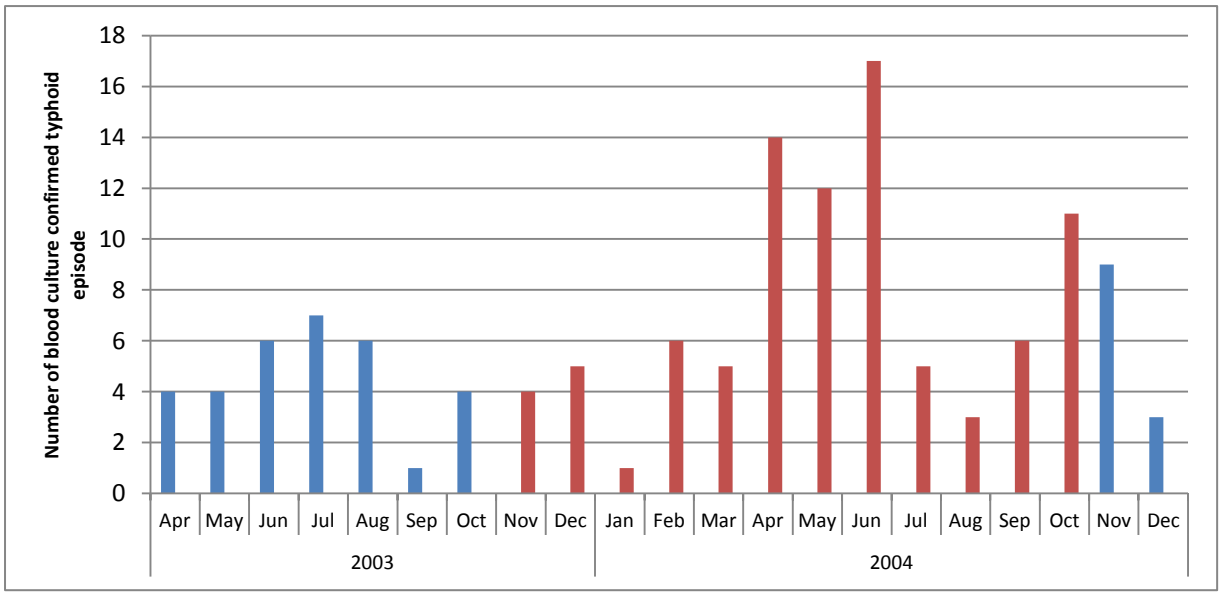
A 12-month period was taken from the pre-vaccination phase to determine the annual incidence rate, rather than annualizing the whole period. The period, from November 2003, was chosen because that time the surveillance system was functioning optimally. A patient from the target population presenting to a participating treatment centre with a history of fever lasting ≥3 days was considered as having a febrile episode. A typhoid episode was defined as an episode of febrile illness during which *S.* Typhi was isolated from a blood culture. Census and surveillance data were double-entered into a custom data entry program (FoxPro, Microsoft, Washington, USA) with error and consistency check programs. Typhoid patients and not "episodes" were considered in the analysis; thus, each individual could be included only once in the numerator for calculation of incidence. We assumed that each person residing in the study area at the time of the census contributed 12 months to the denominator. Incidence per 100,000 person-years was calculated using age-specific denominators from the baseline census. The numerator was the number of age-specific disease episodes. To estimate the prevalence of the disease, we considered the number of episodes per all febrile episodes registered. We calculated 95% confidence intervals for incidence using the Wilson score method. Categorical variables were compared using the χ^2 test and dimensional variables were compared using analysis of variance. A value of $P<0.05$ (two-tailed) was considered statistically significant. Stata/SE 8.0 (Stata Corporation, Texas, USA) was used for the analysis.

Results

There were 4,378 episodes of fever from the community through the surveillance system during the specific 12-month preparation phase (1/Nov/2003 to 30/Oct/2004), of which, 4,342 episodes had blood samples taken. 122 samples yielded *S.* Typhi (Figure 3-1). The incidence rate of *S.* Typhi was 214.2/100,000 persons per year, where the rate was highest in the age group 5 to 15 years (493.5/100,000 persons per year), followed by the age group 2 to 5 years (340.1/100,000 persons per year). Prevalence of the disease was also highest among 5 to 15 years of age at 52.5 cases per 1,000 febrile episodes per year. All *S.* Typhi isolates were tested for antimicrobial agent susceptibility. Multidrug resistance (resistant to 3 first-line antimicrobials agents: chloramphenicol, ampicillin and TMP-SMX) was observed from 8/113

(7%) isolates. Nalidixic acid resistance was found in 65/113 (58%) isolates. One *S.* Typhi isolate (0.9%) was resistant to ciprofloxacin.

Figure 3-1: Blood culture confirmed S. Typhi cases from Kolkata, India during pre-vaccination surveillance period



Red bars denote the typhoid cases from the period used to assess the typhoid disease burden. The period from November 2003 was taken as it took approximately 6 months to ensure the surveillance was optimal.

3.4 Methods

3.4.1 Baseline census and definition of clusters

Prior to vaccination, from February to April, 2004, a census of the *de jure* population was conducted to enumerate all households and individuals in the field trial area. In addition to collecting identifying and demographic characteristics of individuals and households, the census obtained information on socioeconomic characteristics and the type of water and sanitation used by each household. Each household and individual in the census was assigned a unique study identification number and was given a census identification card that provided this number. The census also used geographic positioning system (GPS) tools to record the locational coordinates of each household. The census and geographical mapping was then used to define the 80 contiguous geographical clusters that served as the units of randomization (Figure 3-2). The populations of the clusters ranged from 539 to 1,098.

Figure 3-2: Map of the study area showing the project health outposts and vaccine assignment for the 80 randomized clusters for the Vi Effectiveness Trial in Kolkata, India

These 80 clusters were randomized to receive Vi polysaccharide (red) or inactivated Hepatitis A vaccine (blue).



3.4.2 Compared agents

Vi polysaccharide vaccine (Typherix®, GlaxoSmithKline) was the principal vaccine under study. Each vial contained 25ug of Vi polysaccharide in a single-dose suspension, administered by intramuscular injection in the deltoid region. The control vaccine was inactivated hepatitis A vaccine (Havrix®, GlaxoSmithKline). This vaccine contained 720IU inactivated hepatitis A virus for children (ages 2 to 18 years) and 1440IU for adults in a single-dose suspension, also administered by intramuscular injection in the deltoid region. These vaccines were donated by GlaxoSmithKline. It was not possible to arrange for the two agents to have identical presentations (the Vi polysaccharide vaccine was provided in single-dose syringes; the hepatitis A vaccine was provided in single-dose vials). To decrease the likelihood that these different presentations would create a bias, the compared agents were provided with identical labels giving only a code letter. Four code letters were used: two for children aged 2 to 18 years and two for older persons. In each age stratum one code was used for Vi polysaccharide vaccine and one was used for hepatitis A vaccine; in each cluster two code letters were assigned, one to children and one to adults. Each vaccination centre provided one type of vaccine, thus participants were not exposed to the two different presentations. Members of the project staff involved with vaccine administration and surveillance were not informed of the identities of the codes. Additionally, vaccination teams administering the vaccines were assigned only to administer only one of the vaccines throughout the immunization campaign (*vide infra*).

3.4.3 Randomization

Assignment of the clusters to the two vaccines was stratum-randomized and blocked to ensure balanced numbers of clusters within each stratum. Because earlier surveillance had identified both age and ward of residence as predictors of the risk for typhoid in the study setting, clusters were first stratified by the absolute number of residents aged ≤18 years (<200 versus ≥ 200 persons) and of older residents (<500 versus ≥ 500 persons), creating four strata. Within each stratum, a table of random numbers was used to randomize each of the 80 clusters to one of the two vaccines (Figure 3-2).

3.4.4 Vaccination

The vaccines were administered between November 27 and December 31, 2004. Individuals were eligible for receipt of the vaccine assigned to the cluster of residence if: 1) they were 24 months or older of age at the time of vaccination; 2) they had an axillary temperature ≤37.5 °C if subjective fever was reported; and 3) they were not pregnant or lactating. Vaccination was done by 20 teams, each consisting of a physician, a vaccinator, a recorder, and two assistants. Each administered dose of vaccine was entered into pre-printed vaccine record books, listing each resident by household. All vaccinees were observed for 30 minutes following dosing to detect and manage immediate adverse events. Vaccination was supervised by a supervisor assigned to two or three teams, and external observers monitored and documented several features (consent process, injection safety, data recording, and cold chain) on designated forms. Vaccination was undertaken in special vaccination centres, one for each cluster, and the schedule of vaccination was arranged so that vaccination with Vi polysaccharide vaccine and hepatitis A vaccine was temporally balanced.

Prior to the vaccine campaign, social mobilization was done through the Imams of the local mosques, private physicians from the area, and local influential persons. Additionally, local social scientists organized community sessions to inform community members about the trial. The day before the vaccination of a cluster, cluster households were visited to provide information about the trial and were given household ID cards that included the site, date, and time for vaccination. In addition, individuals visiting vaccination posts on the day of immunization were given information about the nature of the trial, expected risks and benefits, and procedural details as part of the informed consent. Upon agreeing to participate, a thumbprint or signature was affixed on the vaccination record book together with the signature of a witness.

After the conclusion of surveillance, age-eligible residents of clusters originally assigned to Vi polysaccharide vaccine were offered a dose of hepatitis A vaccine, and age-eligible persons originally assigned to hepatitis A vaccine were offered a dose of Vi polysaccharide vaccine.

3.4.5 Surveillance for adverse events

All subjects were observed for immediate adverse events. A subset of 320 subjects, two from subjects administered each code letter assigned to the cluster, was selected for daily home follow-up for three consecutive days after vaccination to detect local and systemic adverse events. 202 agreed to be followed up, after acquisition of written informed consent. We did not replace those who did not take the vaccine or did not consent to provide the information (n=118). Passive surveillance was implemented for all patients from the study area who presented for care at the 5 study clinics (*vide infra*). In addition, physicians at the Infectious Disease and B.C. Roy Hospitals, the only two public hospitals serving the study population, were requested to report any admission of a patient known to have received one of the study vaccines. Finally, all deaths occurring in the study population during this same period were monitored by local community health workers who visited each household monthly, and verbal autopsies were conducted. All adverse events following immunization, deaths, and verbal autopsies, all of them causal or not causal, were entered onto special study data forms by physicians, which also included physician assessments as to whether the noted adverse event could have been causally related to one the vaccines tested in trial. Serious adverse events, defined as in ICH GCP guideline (International Conference on Harmonization 1996), were monitored by a local clinical monitor; the Data and Safety Monitoring Board; the study secretariat at the International Vaccine Institute; and the manufacturer.

3.4.6 Surveillance for typhoid fever

To detect enteric fever in the study population, several strategies were employed. Firstly, 5 study clinics were established by NICED in the two study wards, three in Ward 29 and two in Ward 30. These clinics gave routine care for all outpatient visits, and referred patients with severe disease for hospital care. Private medical care providers in the two study wards were encouraged to refer patients to these study clinics for diagnostic evaluations. Second, the emergency rooms, outpatient clinics, and inpatient wards of the Infectious Disease Hospital

and B.C. Roy Hospital monitored all patients from the study area presenting with febrile illnesses. In each of these treatment settings all subjects from the study area presenting with a history of at least 3 days of fever were examined by a study physician, data on the subject's history and physical findings were systematically entered onto a structured clinical case report form, after obtaining verbal informed consent, and a blood specimen was collected for culture. For the blood culture, approximately 5 to 8 ml of blood was collected to inoculate Bactec Plus F/ Aerobic® culture bottles (Becton Dickinson, New Jersey, USA); for younger children, about 3 to 5 ml of blood was inoculated into Bactec Peds Plus® culture bottles (Becton Dickinson, New Jersey, USA).

Several methods were used to help identify subjects seen in the surveillance. Subjects were encouraged to bring study identification cards issued during the census when presenting for care. In addition, in all of the treatment sites a computerized version of the most recent census of the population was used to identify each subject, assisted by a special search algorithm developed for the study. These data sources allowed treatment centre staff to locate the subject's census number for later linkage with the vaccination database at the conclusion of the study. The vaccine code received by subjects was recorded in a separate database and kept secretly in Seoul to discourage ongoing tabulations of cases by code letter. Finally, whenever a blood culture yielded either *S.* Typhi or *S.* Paratyphi, a study team was dispatched to the home of the patient within seven days to verify that the subject whose name was given at the treatment site had indeed visited the treatment site for care on the date noted in the surveillance.

3.4.7 Laboratory procedures

Laboratories for microbiological evaluations of blood cultures collected by the project were set up at each site. Approximately 5 to 8 ml of blood was collected from adults and used to inoculate Bactec Plus Aerobic® culture bottles (Becton Dickinson, New Jersey, USA); for children, 3 to 5 ml of blood was inoculated into Bactec Peds Plus® culture bottles (Becton Dickinson, New Jersey, USA). Blood culture bottles were transported to the laboratory the

same day of the sampling, incubated, and observed for growth. When bacterial growth was confirmed, the blood culture bottle was sub-cultured on MacConkey agar plate. In the absence of visible growth, the bottle was sub-cultured on MacConkey agar on days 1, 2, 4 and 7. The bottles were incubated for a minimum of 10 days before discarding. Lactose non-fermenting colonies from MacConkey agar were screened using Kligler's iron agar (KIA). The colonies suggestive of S. Typhi (acid butt, alkaline slant, trace amount of hydrogen sulfide) were subjected to slide agglutination with Vi antiserum, Salmonella D1 group-specific antiserum, Factor 9 O antiserum and factor d H antiserum as appropriate (Bopp, Brenner et al. 1999). All presumptive S. Typhi isolates were sent to a reference laboratory (Wellcome Trust Research Unit, Ho Chi Minh City, Vietnam) for confirmation. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method on Muller-Hinton agar with standard antimicrobial discs (Becton Dickenson Laboratories, New Jersey, USA). Resistance to six antimicrobial agents, chloramphenicol, ampicillin, TMP-SMX (trimethoprim-sulphamethoxazole), nalidixic acid, ciprofloxacin, and ceftriaxone was tested.

3.4.8 Surveillance for demographic events

Following vaccination, two censuses were carried out, the first in October to November 2005, and the second in January to February, 2007. In each census information was obtained on all out-migrations, internal migrations, and deaths among the population present at the time of vaccination. In addition, deaths were monitored by community health workers who visited each residence in their assigned areas every month. Verbal autopsies were performed for deaths within three months after the time of death, by study physicians who used a structured questionnaire for an interview with an adult household member.

3.4.9 Definitions

Several definitions, formulated *a priori*, were required for the analysis of data from the trial. A *household* was defined as all persons residing together and sharing the same cooking pot.

Zero time, denoting the time marking the onset of follow-up, was the date of vaccination for recipients of Vi or hepatitis A vaccines, and, for non-vaccinees (i.e. those who did not consent to receive the vaccine, medically ineligible to receive the vaccine on the vaccination day, or absent on the vaccination day), was the date at which 50% of vaccinees in the same cluster had received a vaccine. Since there were patients seeking care multiple times in a short period of time, defining an episode was considered important so that those visits were concatenated into one illness period. A *febrile episode* comprised treatment visits of patients with subjective or objective (≥38 °C axillary) fever in which the recalled date of onset for fever for one visit was within 14 days of presentation for the next such visit. An episode of *typhoid fever* was a febrile episode in which *S.* Typhi was isolated from at least one blood culture. An episode of *paratyphoid fever* was a febrile episode in which *S.* Paratyphi was isolated from at least one blood culture, but *S.* Typhi was not isolated from any blood cultures. The *onset* of these episodes was taken as the recalled onset date of fever by the patient for her/his first treatment visit of the episode.

3.4.10 Analytic strategies

A detailed analytical plan based on the study protocol was composed prior to conducting any analysis. The analytical plan and associated statistical program was made based on re-randomized data, completely independent of the assigned randomization codes to ensure maintaining the blinding. This analytical plan was submitted to the Data and Safety Monitoring Board for approval. Analytical plan is included as an appendix.

In short, statistical comparison of the two groups for baseline individual-level variables employed Generalized Estimating Equations (GEE) to adjust for membership in the randomized clusters, with the logit link function used for dichotomous variables and the identity link function for dimensional variables (Liang and Zeger 1986). Cluster-level baseline variables were compared using the Student t test for dimensional variables and the chi square test for categorical variables. Evaluation of vaccine safety entailed comparison of recipients of Vi polysaccharide vaccine with those of hepatitis A vaccine for immediate adverse events, for

events ascertained daily for three days after dosing in a subset of participants, for medical events detected in study clinics and hospitals during the month after dosing, and for mortality, including cause-specific mortality, during the month following dosing. Statistical evaluation of these outcomes, which were dichotomous, employed GEE with the logit link function to adjust for cluster allocation (Liang and Zeger 1986).

Vaccine protection was assessed in several ways. In each approach, enteric fever episode outcomes were analyzed if their onset occurred from the day after zero time, and persons were analyzed according to the vaccine assigned to their clusters. The primary objective of the study was to evaluate *total vaccine protection*, comparing rates of episodes of typhoid fever in recipients of Vi polysaccharide vaccine versus recipients of hepatitis A vaccine. As secondary objectives two additional types of vaccine protection were estimated: the *indirect vaccine protection* conferred by Vi polysaccharide vaccine against typhoid fever by comparing the rates of typhoid fever in non-vaccinees in clusters assigned to Vi polysaccharide vaccine versus those for non-vaccinees in the clusters assigned to hepatitis A vaccine; and the *overall vaccine protection* by comparing rates of typhoid fever in all persons residing in clusters assigned to Vi polysaccharide vaccine versus all persons residing in clusters assigned to hepatitis A vaccine (Halloran, Struchiner et al. 1997). Finally, to assess whether bias could have distorted the rates of typhoid fever in the Vi polysaccharide vaccine versus the hepatitis A vaccine clusters, the study evaluated total protection by Vi polysaccharide vaccine against episodes of paratyphoid fever (all of which were due to *S.* Paratyphi A in the study), a disease for which protection by Vi polysaccharide vaccine was not anticipated but which has clinical characteristics closely resembling typhoid fever. In all analyses, we evaluated vaccine protection against first episodes of the disease outcome during surveillance after zero time.

To estimate vaccine protection, Cox proportional hazard regression models was used, verifying first that the proportionality assumption was satisfied for all independent variables (Lin, Wei et al. 1993). These models compared the time from zero time to the first episode of the analyzed outcome in members of the Vi polysaccharide vaccine group versus members of the hepatitis A vaccine group; censoring events for these analyses included migration out of the zero-time cluster, death, or completion of the two-year, post-zero time follow-up,

whichever came first. These were ascertained through censuses conducted during the follow up period. Relative rates (RRs) of the target outcome in the Vi versus the hepatitis A groups were estimated by exponentiating the coefficient for the vaccine variable in these models, and vaccine protective effectiveness (PE) was estimated as (1- RR) X 100%. Standard errors for the coefficients for the vaccine variable in the models were used to estimate P values and 95% confidence intervals for the RRs. Simple analyses of vaccine impact were adjusted for the design effect of the cluster randomization in these models with robust sandwich variance estimates, also known as Huber-White standard error estimates (Reid and Crèpeau 1985; Lin and Wei 1989). Final adjusted estimates were obtained from models that also included the variables used for stratified randomization, as well as potentially confounding variables independently associated with the time to the event at $P<0.1$ in a backward selection algorithm. Finally, to evaluate heterogeneity of vaccine protection among different subgroups of participants, interaction terms were evaluated between the vaccination and subgroup variables in these models. All P values and 95% confidence intervals for these analyses were estimated in a two-tailed fashion.

3.4.11 Sample size requirement

The sample size required for the primary analysis of total vaccine protection assumed vaccine PE ≥ 60% during two years of follow-up; two treatment arms of equal size, each with 40 clusters; an incidence of typhoid in the hepatitis A vaccine group of ≥ 2 cases per 1,000 persons; a coefficient of variation for disease incidence among clusters of .5; 80% power; and assessment of PE at $P<.05$ (two-tailed). With these assumptions, the required sample size was 20,757 per group (Hayes and Bennett 1999).

3.4.12 Monitoring and ethics

A Data and Safety Monitoring Board specially constituted for the trial approved the study protocol, monitored serious adverse events during the study, and verified that the dataset was

frozen and the analytic plan was finalized prior to analyses of the data. Institutional Review Boards of the International Vaccine Institute, the National Institute of Cholera and Enteric Diseases, and the Indian Council of Medical Research approved the protocol for the study and monitored the progress of the study. Information regarding the study was provided first to the community influential persons (i.e. community leaders, imams, and political members) for their consent, followed by community information sessions. During the time of the vaccination, informed consent was obtained from all participants and recorded in the vaccination record book in a form of written signature or thumbprint (see appendix for vaccination procedure and information provided to obtain consents).

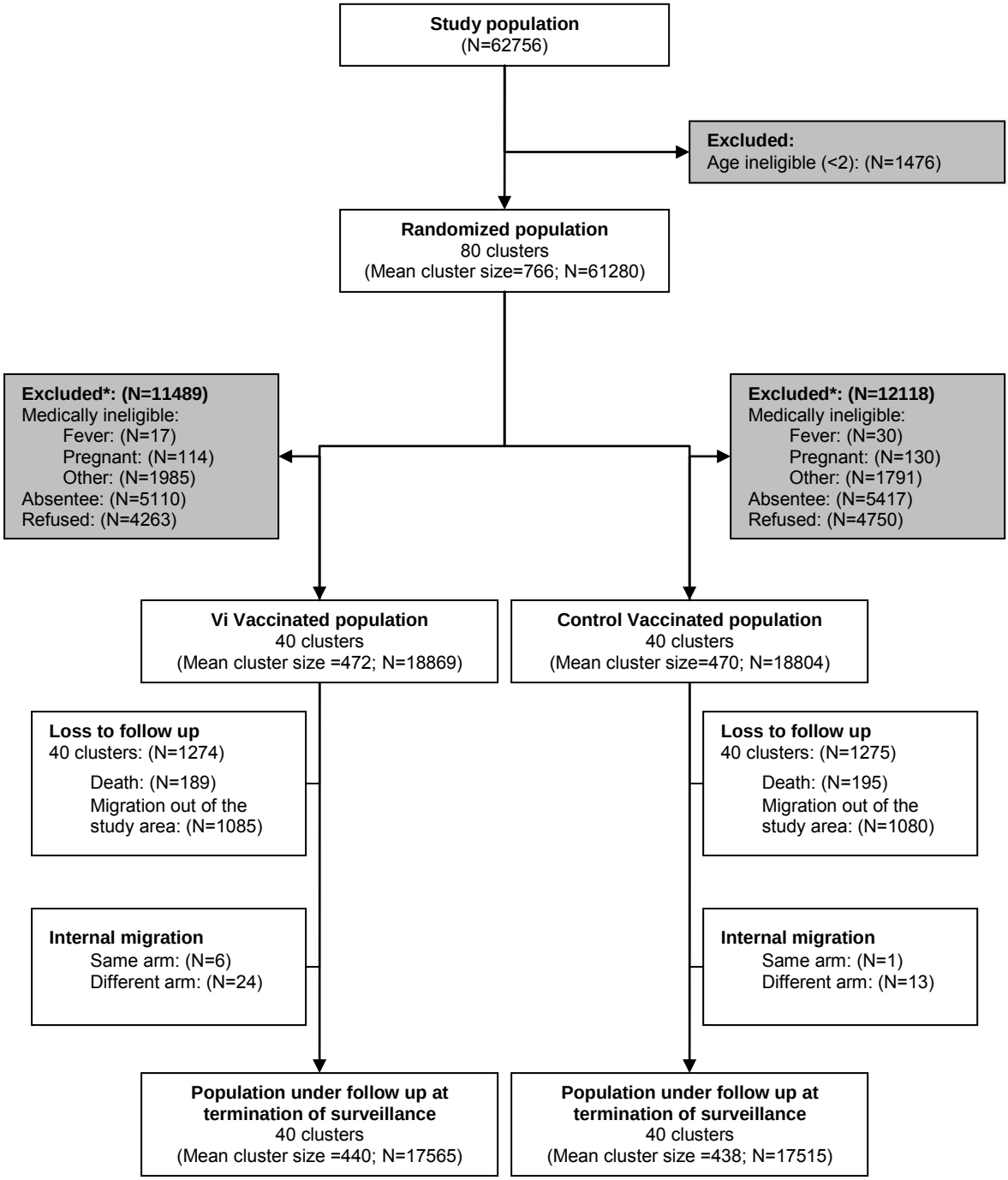
No agreements were signed regarding confidentiality of the data among donors, the study sponsor (the International Vaccine Institute), the vaccine manufacturer, and the investigators. The vaccine manufacturer donated the vaccines and conducted serologic assays in a blinded manner but did not participate in the study design or statistical analysis.

3.5 Results

3.5.1 Assembly of the study population

At zero-time there were 62,756 subjects residing in the 80 trial clusters, of whom 61,280 were age-eligible for the trial (Figure 3-3). As shown in the figure, 37,673 persons received a dose of Vi or hepatitis A vaccine, 18,869 in the Vi polysaccharide vaccine group and 18,804 in the hepatitis A vaccine group. During the two years of follow-up after zero time, 2,549 (7%) of vaccinees died or were lost to follow-up. A further 44 (0.1%) subjects migrated out of their zero-time clusters to another cluster in the study.

Figure 3-3: CONSORT chart of the study population of the Kolkata Vi Effectiveness Trial



***Definitions of exclusions:**
Fever – acute febrile (axilliary ≥38ºC) persons on the day of vaccination
Pregnant – persons being pregnant on the day of vaccination
Other – persons with other medical conditions such as previous history of allergy to vaccination, diabetes, and other chronic conditions which the attending vaccination team doctor considered ineligible to receive the vaccine on the day of vaccination
Absentee – persons who were not found on the day of vaccination
Refused – persons who either did not consent to receive the vaccine or refused to come to the vaccination centre

3.5.2 Comparability of the compared groups at zero time

Table 3-1 and Table 3-2 compare vaccinated individuals in the 40 Vi polysaccharide vaccine clusters with those in the 40 hepatitis A vaccine clusters. All dosed subjects received the vaccine assigned to the clusters. The tables show that the two treatment groups were well balanced at both the individual and cluster levels with respect to a variety of demographic, socioeconomic, and water-hygiene characteristics. Vaccine coverage of clusters assigned to Vi polysaccharide vaccine (61%) was nearly identical to that for clusters assigned to hepatitis A vaccine (60%).

Table 3-1: Characteristics of individuals at zero time, Kolkata. India

Variables	Vaccine group		p-value*
	Vi (n=18,869)	Hep A (n=18,804)	
Mean (SD)† age (years)	28.54 (17.96)	27.89 (17.76)	0.55
No. (%) of males	9,876 (52.34)	9,920 (52.75)	0.98
No. (%) of Hindus[1)]	12,335 (65.37)	10,825 (57.57)	0.53
No. (%) of individuals living in a household with literate household head	13,980 (74.09)	13,099 (69.66)	0.18
No. (%) of individuals living in a household with household head having more than 5 years of schooling	10,070 (53.37)	10,033 (53.36)	0.79
No. (%) of individuals living in a household with important economic contributor having stable occupation[2)]	3,570 (18.93)	3,603 (19.16)	1.00
No. (%) of individuals living in a household using safe toilet[3)]	905 (4.81)	577 (3.08)	0.18
No. (%) of individuals living in a household using safe water source (tap at household level)	2,706 (14.35)	1,792 (9.53)	0.20
No. (%) of individuals living in a household using safe water source (tap or hand pump at household level)	2,711 (14.37)	1,824 (9.70)	0.22
No. (%) of individuals living in a household using boiled or filtered water	1,635 (8.67)	1,191 (6.33)	0.17
No. (%) of individuals living in a household always wash hands with soap and water after defecation	10,991 (58.28)	14,134 (75.16)	0.05
No. (%) of individuals living in a household having specific place for waste disposal	18,547 (98.54)	18,429 (98.08)	0.82
No. (%) of individuals living in owned house	5,155 (27.33)	5,297 (28.19)	0.98
No. (%) of individuals living in a household owning refrigerator	3,048 (16.15)	2,904 (15.44)	0.69
No. (%) of individuals living in a household owning motorbike	1,059 (5.61)	859 (4.57)	0.23
No. (%) of individuals living in a household owning telephone	2,705 (14.34)	2,568 (13.66)	0.61
No. (%) of individuals living in a household owning all luxury items[4)]	632 (3.35)	515 (2.74)	0.30
No. (%) of individuals living in a household owning at least one luxury item[4)]	4,131 (21.89)	3,918 (20.84)	0.58
Mean (SD) † monthly household expenditure (Indian Rupee)	3,562.97 (2,080.3)	3,442.30 (2,329.6)	0.37
No. (%) of individuals having high monthly household expenditure[5)]	7,743 (41.55)	7,457 (39.90)	0.41
Mean (SD) † monthly per-capita expenditure[6)] (Indian Rupee)	583.87 (379.87)	563.06 (375.05)	0.53
No. (%) of individuals having high monthly per-capita expenditure[5)]	7,795 (41.83)	7,636 (40.86)	0.69
No. (%) of age eligible individuals for the trials living in a larger cluster[7)]	8,510 (45.10)	9,661 (51.38)	0.50
Mean (SD) † household size (all individuals at *zero-time*)	7.12 (3.87)	7.05 (3.65)	0.82
Mean (SD) † distance (m) from household to the nearest health outpost	152.41 (79.68)	158.81 (78.47)	0.70
No. (%) of individuals living in household longer distance to health outpost[8)]	8,900 (47.17)	9,935 (52.83)	0.58
No. (%) of individuals living in lager cluster size of adults at *zero-time* stratification[9)]	11,820 (62.64)	10,368 (55.14)	0.50
No. (%) of individuals living in lager cluster size of paediatrics at *zero-time* stratification[10)]	10,667 (56.53)	11,409 (60.67)	0.65

[*] The p-values are derived by comparing the differences between the two groups adjusted for cluster effects (Using GEE with the logit link function for dichotomous variables and the identity link function for dimensional variables).

[†] Mean and Standard Deviation (SD) was adjusted for cluster effects.

[1)] Hindus compared to non-Hindus (Muslims, Christians, others)

[2)] Stable occupation: professional, service, shop owner, or farm owner

[3)] Safe toilet: use of own (non-shared) flush toilet

[4)] Luxury items: Refrigerator, motorbike, and/or washing machine

[5)] Higher (household/per-capita household) expenditure: More than the median household/per-capita household expenditure; median: median individual vaccinee

[6)] Per-capita household expenditure: calculated from total household expenditure divided by the number of active members in the household prior to vaccination (1USD = 45 Indian Rupees); median: median individual vaccinee

[7)] Larger cluster: More than the median cluster; median: median individual vaccinee (not median cluster)

[8)] Longer distance to the nearest health outpost: more than the median distance to the nearest health outpost; median: median individual vaccinee

[9)] Larger cluster size of adults (age>18 years) at *zero-time* stratification: ≥500 adults per cluster at stratification.

[10)] Larger cluster size of paediatric population (age≤18 years) at *zero-time* stratification: ≥200 paediatrics per cluster at stratification.

Table 3-2: Characteristics of the clusters at *zero time*, Kolkata, India.

Randomized population (n=37,673) unless otherwise noted

Variables	Vi (clusters=40)	Hep A (clusters=40)	Total (clusters=80)	p-value[†]
Cluster stratification:				
<500 adults and <200 children	4	6	10	0.76
<500 adults and ≥200 children	13	14	27	
≥500 adults and <200 children	14	10	24	
≥500 adults and ≥200 children	9	10	19	
Mean (SD; min., max.) number of children (2-18)	255.90 (117.50; 117.00, 506.00)	273.03 (115.42; 131.00, 508.00)	264.46 (116.04; 117.00, 508.00)	0.51
Median number of children (2-18)	220.50	217.00	217.00	0.43
Mean (SD; min., max.) number of Adults (19+)	503.05 (84.47; 287.00, 694.00)	500.03 (93.16; 319.00, 734.00)	501.54 (88.37; 287.00, 734.00)	0.88
Median number of Adults (19+)	510.50	504.00	510.00	0.63
Mean (SD; min, max) mean age of persons in cluster	29.05 (5.03; 22.08, 36.21)	28.47 (4.60; 22.10, 36.07)	28.76 (4.80; 22.08, 36.21)	0.59
Median mean age of persons in cluster	28.79	27.67	28.09	0.59
Mean (SD; min., max.) number of households	142.03 (27.18; 72.00, 208.00)	145.58 (36.10; 84.00, 244.00)	143.80 (31.80; 72.00, 244.00)	0.62
Median number of households	142.50	140.00	142.00	0.81
Mean (SD; min., max.) number of total individuals	776.88 (135.53; 539.00, 1098.00)	792.03 (141.62; 550.00, 1081.00)	784.45 (137.94; 539.00, 1098.00)	0.63
Median number of total individuals	761.00	763.50	761.50	0.59
Mean (SD; min., max.) number of age eligible individuals	758.95 (128.57; 525.00, 1062.00)	773.05 (134.66; 544.00, 1031.00)	766.00 (131.01; 525.00, 1062.00)	0.63
Median number of age eligible individuals	745.00	743.50	745.00	0.63
Mean (SD; min., max.) number of dosed individuals	471.73 (102.97; 222.00, 645.00)	470.10 (104.20; 208.00, 704.00)	470.91(102.93; 208.00, 704.00)	0.94
Median number of dosed individuals	479.50	469.50	473.50	0.89
Mean (SD; min., max.) total study population density/10m^2	18.44 (17.78; 1.85, 69.38)	22.28 (20.08; 2.84, 87.49)	20.36 (18.94; 1.85, 87.49)	0.37
Median total study population density/10m^2	9.75	12.58	11.10	0.47
Mean (SD; min., max.) pre-vaccination incidence of typhoid fever	1.54 (1.40; 0.00, 6.25)	1.38 (1.38; 0.00, 4.91)	1.46 (1.39; 0.00, 6.25)	0.59
Median pre-vaccination incidence of typhoid fever	1.43	1.16	1.32	0.45
Mean (SD; min., max.) of vaccine coverage	60.98 (10.53; 37.50, 78.75)	60.09 (11.51; 21.42, 76.94)	60.54 (10.97; 21.42, 78.75)	0.72
Median of vaccine coverage	62.37	62.31	62.37	0.81

[†]The p-values are derived by comparing the differences between the two groups (chi-square test for categorical variables, t-test for mean comparison, non-parametric method (Wilcoxon rank sum test) for median comparison).

3.5.3 Evaluation of adverse events following vaccination

During the 30-minute period of observation after injection with the assigned vaccine, 4 events were noted: 3 in the Vi polysaccharide vaccine group and 1 in the hepatitis A group. All were syncopal episodes (fainting) or having a sense of vertigo, and none was judged to be a serious adverse event, nor an event causally related to receipt of vaccine.

202 subjects were followed up for three consecutive days after dosing, 92 in the Vi polysaccharide vaccine group and 110 in the hepatitis A vaccine group to assess adverse event in solicited manner (Table 3-3). Except for one subject in the hepatitis A vaccine group, who could not be located on days 2 and 3, all subjects were examined on each of the three days of follow-up. Over the entire three-day period, several signs and symptoms were significantly more common in the hepatitis A group than in the Vi group: redness (22% vs. 4%, P<.001) and pain (55%, vs. 18%, P<.001) at the injection site, fever (7% vs. 1%, P<.05), and fatigue (18% vs. 4%, P<.01). No signs or symptoms were significantly more common in the Vi group than in the hepatitis A group.

During the 30 days following dosing, 18 deaths (8 in the hepatitis A vaccine group and 10 in the Vi polysaccharide vaccine group) and one non-fatal serious adverse event (a patient with a subarachnoid haemorrhage in the hepatitis A vaccine group) were reported among recipients of either vaccine (Table 3-4). 11 of the 18 deaths were due to cardiovascular causes and none of the events was judged to be causally related to receipt of vaccine.

Table 3-3: Distribution of solicited adverse event symptoms by vaccine received

The p-values were derived by comparing the difference between two groups using chi-square test for categorical variables, except when data are sparse Fisher's exact test was used, and t-test for continuous variable.

	Day 1			Day 2			Day 3			Any day		
	Vi (n=92)	Hep A (n=110)	P-value	Vi (n=92)	Hep A (n=109)	P-value	Vi (n=92)	Hep A (n=109)	P-value	Vi (n=92)	Hep A (n=110)	P-value
Redness	4	24	0.001	0	4	0.125	0	0	-	4	24	0.000
Swelling	4	10	0.270	1	1	1.000	0	1	1.000	4	10	0.267
Pain	15	55	0.000	6	19	0.018	0	10	0.005	17	60	0.000
Fever	0	4	0.130	1	4	0.375	0	1	1.000	1	8	0.042
Fatigue	1	17	0.000	4	9	0.388	2	4	0.695	4	20	0.004
headache	4	14	0.047	3	9	0.145	1	6	0.136	6	16	0.069
Gastrointestinal	1	1	1.000	2	2	1.000	1	1	1.000	4	2	0.414
General aches	5	7	1.000	2	8	0.111	0	6	0.035	5	15	0.052

Table 3-4: Distribution of deaths during 30 days on or after vaccination by vaccine received

Cause of death	Vi	Hep A	Total
Acute myocardial inaction	1	1	2
Acute myocardial infarction, unspecified	1		1
Essential (primary) hypertension		1	1
Ill-defined and unknown cause of mortality (it was “missing”)		1	1
Intracerebral haemorrhage	1		1
Malnutrition-related diabetes mellitus	1		1
Other diseases of digestive system		1	1
Burn and corrosion, body region unspecified (it was “Other shock”)	1		1
Plasmodium falciparum malaria, unspecified		1	1
Poisoning by and exposure to other and unspecified chemlcals and noxious substances, undetermined intent	1		1
Pulmonary heart disease, unspecified		1	1
Respiratory tuberculosis unspecified, without mention of bacteriological or histological confirmation	1		1
Sequelae of cerebrovascular disease	1		4
Stroke, not specified as haemorrhage or infarction	2	2	1
Total	**10**	**8**	**18**

3.5.4 Evaluation of total protection conferred by Vi polysaccharide vaccine against typhoid fever

Typhoid fever occurred throughout the follow-up period, with no consistent seasonality, though increases in the caseload occurred in the autumn of 2005 and summer months of 2006. This can be seen from the Kaplan-Meier curve and from the graph showing the occurrence of first episodes of typhoid fever among recipients of either vaccine during the two years of follow-up (Figure 3-4 and Figure 3-5).

Table 3-5 shows the overall occurrence of first episodes of typhoid in recipients of Vi polysaccharide vaccine vs. recipients of hepatitis A vaccine. There were 96 episodes of typhoid fever in the hepatitis A vaccine group, as compared with 34 episodes in the Vi polysaccharide vaccine group (PE= 61%, 95% CI: 41%, 75%, P<.0001 in adjusted model). When evaluated by age group at vaccination in adjusted models, Vi polysaccharide vaccine conferred 80% (95%CI: 53%, 91%, P<.001; Table 3-6) protection to children vaccinated at ages under five years, 56% (95%CI: 18%, 77%, P<.01; Table 3-7) protection to persons given Vi polysaccharide vaccine at ages between five years and less than 16 years, and 46% (95%CI: -43%, 79%, P=.21; Table 3-8) protection to persons given Vi polysaccharide vaccine at ages 16 years and older in adjusted models. The difference in Vi protection by age was not statistically significant (P=.11).

Figure 3-4: Occurrence of first episodes of culture confirmed typhoid fever by month during the follow up period for the Kolkata Vi effectiveness trial

Vaccination took place in December 2004.

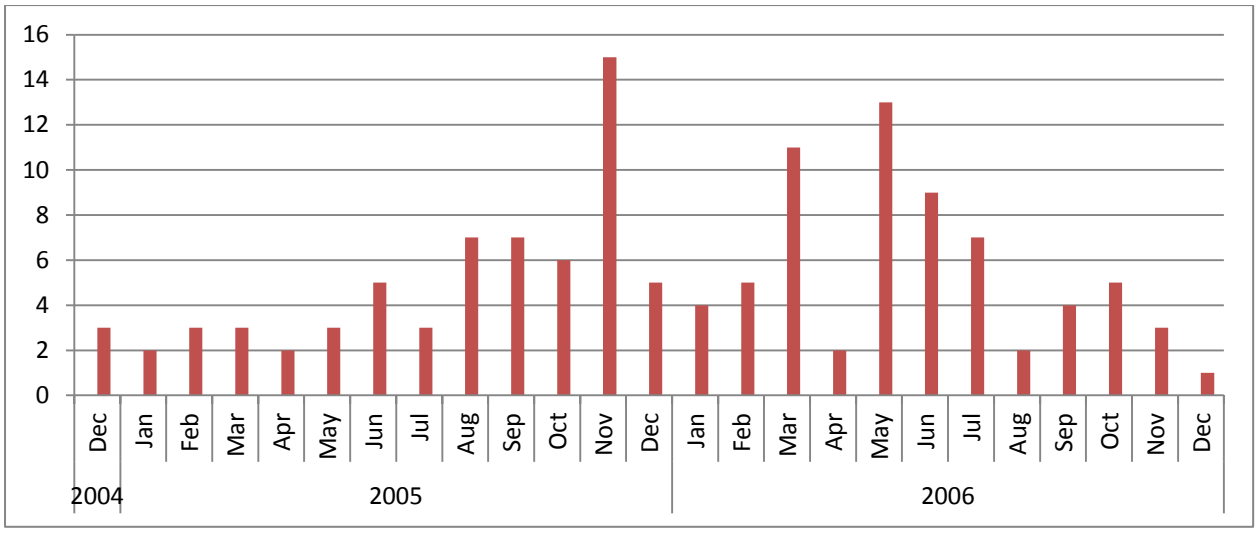


Figure 3-5: Kaplan Meier Curve of the Kolkata Vi effectiveness trial followed up for two years

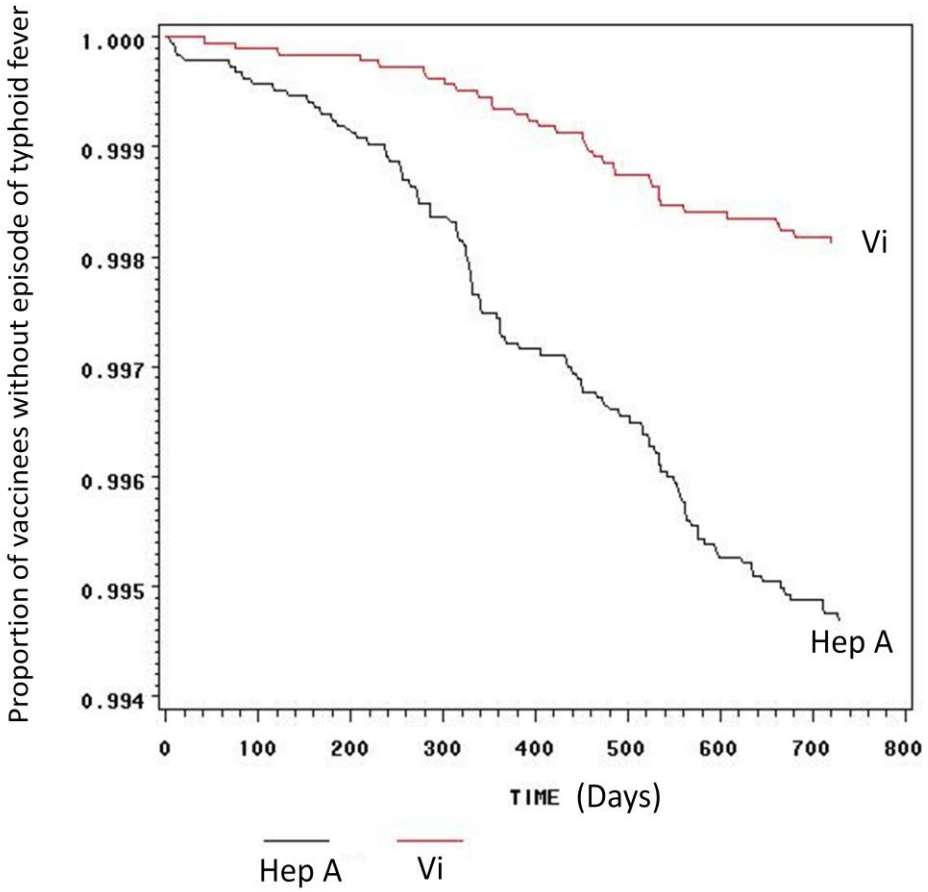


Table 3-5: Vaccine protection against typhoid in Kolkata, India, amongst all vaccinees (Total Protection)

Randomized population (n=37,673)

Events	Frequency		Rate*	
	Vi (n=18,869)	Hep A (n=18,804)	Vi	Hep A
Typhoid cases[†]	34	96	0.2555	0.7265
Migration-out of study area	1112	1085	8.3550	8.2105
Deaths	189	194	1.4201	1.4681
Person-days[§]	13309337	13214761		
Average of Person-days	705.3547	702.7633		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.3517	0.2120	0.5835	0.0001

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.3857	0.2509	0.5929	0.0000
Larger cluster size of paediatrics at stratification[10)]	0.7269	0.3703	1.4269	0.3540
Larger cluster size of adults at stratification[9)]	1.2573	0.7883	2.0053	0.3364
Individuals living in a household having specific place for waste disposal	0.4649	0.2202	0.9817	0.0446
Individuals with high per-capita monthly expenditure[4)]	0.6119	0.4018	0.9320	0.0221
Hindus (0:others; 1:hindu)	0.4608	0.2330	0.9110	0.0259
Age (years)	0.8935	0.8703	0.9172	0.0000

[†]Refer to footnotes of table in the previous section for description of the variables.

Table 3-6: Vaccine protection against typhoid in Kolkata, India, amongst all vaccinees aged 2 to 4 years (Total Protection)

Randomized population (n=2,192)

Events	Frequency		Rate*	
	Vi (n=1,097)	Hep A (n=1,095)	Vi	Hep A
Typhoid cases[†]	5	27	0.6419	3.5441
Migration-out of study area	53	67	6.8045	8.7946
Deaths	2	-	0.2568	-
Person-days[§]	778893	761832		
Average of Person-days	710.0210	695.7370		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.1812	0.0774	0.4242	0.0001

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.2025	0.0871	0.4706	0.0002
Hindus (0:others; 1:hindu)	0.3233	0.1091	0.9583	0.0417
Individuals with high per-capita monthly expenditure[4)]	0.3632	0.1311	1.0064	0.0514
Larger cluster size of adults at stratification[9)]	1.1470	0.6429	2.0462	0.6424
Larger cluster size of paediatrics at stratification[10)]	0.3322	0.1062	1.0392	0.0582

[†]Refer to footnotes of table in the previous section for description of the variables.

Table 3-7: Vaccine protection against typhoid in Kolkata, India, amongst all vaccinees aged 5 to 15 years (Total Protection)

Randomized population (n=8,866)

Events	Frequency		Rate*	
	Vi (n=4,282)	Hep A (n=4,584)	Vi	Hep A
Typhoid cases[†]	21	54	0.6920	1.6697
Migration-out of study area	235	244	7.7440	7.5446
Deaths	8	4	0.2636	0.1237
Person-days[§]	3034593	3234119		
Average of Person-days	708.6859	705.5233		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4145	0.2105	0.8162	0.0109

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4360	0.2325	0.8175	0.0097
Larger cluster size of paediatrics at stratification[10)]	0.7144	0.3166	1.6118	0.4179
Larger cluster size of adults at stratification[9)]	1.4348	0.7837	2.6266	0.2420
Hindus (0:others; 1:hindu)	0.3322	0.1417	0.7788	0.0112
Individuals living in a household having specific place for waste disposal	0.3223	0.1417	0.7333	0.0069

[†]Refer to footnotes of table in the previous section for description of the variables.

Table 3-8: Vaccine protection against typhoid in Kolkata, India, amongst all vaccinees aged 16 years and above (Total Protection)

Randomized population (n=26,615)

Events	Frequency		Rate*	
	Vi (n=13,490)	Hep A (n=13,125)	Vi	Hep A
Typhoid cases[†]	8	15	0.0842	0.1627
Migration-out of study area	824	774	8.6775	8.3959
Deaths	179	190	1.8850	2.0610
Person-days[§]	9495851	9218810		
Average of Person-days	703.9178	702.3855		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.5181	0.1869	1.4360	0.2061

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.5421	0.2050	1.4338	0.2173
Larger cluster size of paediatrics at stratification[10)]	2.4238	0.8594	6.8361	0.0942
Larger cluster size of adults at stratification[9)]	0.8552	0.3041	2.4050	0.7668

[†]Refer to footnotes of table in the previous section for description of the variables.

3.5.5 Assessment of indirect and overall protection by Vi polysaccharide vaccine against typhoid fever

The level of vaccine coverage of the clusters was 61% (61% in the Vi polysaccharide vaccine clusters and 60% in the hepatitis A vaccine clusters). To evaluate indirect protection of this level of coverage by Vi polysaccharide vaccine against typhoid fever, we compared the incidence of typhoid fever among non-recipients of Vi in clusters assigned to Vi, with the incidence of typhoid among non-recipients of hepatitis A vaccine in clusters assigned to this vaccine. The baseline characteristics of these non-recipients were similar (Table 3-9, Table 3-10). As shown in Table 3-11, simple (PE= 45%; 95%CI: 1%, 70%; P<.05) and adjusted (PE= 44%; 95%CI: 1%, 69%; P<.05) analyses both showed significant indirect protection conferred by Vi polysaccharide vaccine against non-vaccinated persons residing in clusters assigned to Vi polysaccharide vaccine. Overall protection by Vi polysaccharide vaccine was evaluated by comparing typhoid incidence among all members of clusters assigned to Vi polysaccharide vaccine versus all members of clusters assigned to hepatitis A vaccine. The baseline characteristics of these cohorts were similar (Table 3-12, Table 3-13). The overall protection was 60% (95%CI: 39%, 74%; P< .0001) in the simple analysis and 58% (95%CI: 38%, 72%; P< .0001) in the adjusted analysis (Table 3-14).

Table 3-9: Characteristics of the non-vaccinee population at *zero time*, Kolkata, India

Variables	Vaccine group of the cluster		p-value*
	Vi (n=12,206)	Hep A (n=12,877)	
Mean (SD)† age (years)	29.63 (18.75)	29.21 (18.89)	0.72
No. (%) of males	6,804 (55.74)	7,191 (55.84)	0.80
No. (%) of Hindus[1)]	6,459 (52.92)	6,053 (47.01)	0.48
No. (%) of individuals living in a household with literate household head	9,018 (73.88)	8,973 (69.68)	0.33
No. (%) of individuals living in a household with household head having more than 5 years of schooling	6,563 (53.77)	6,609 (51.32)	0.73
No. (%) of individuals living in a household with important economic contributor having stable occupation[2)]	2,588 (21.20)	2,690 (20.89)	0.89
No. (%) of individuals living in a household using safe toilet[3)]	884 (7.43)	672 (5.38)	0.45
No. (%) of individuals living in a household using safe water source (tap at household level)	1,998 (16.37)	1,642 (12.75)	0.34
No. (%) of individuals living in a household using safe water source (tap or hand pump at household level)	2,015 (16.51)	1,651 (12.82)	0.34
No. (%) of individuals living in a household using boiled or filtered water	1,328 (10.88)	1,143 (8.88)	0.39
No. (%) of individuals living in a household always wash hands with soap and water after defecation	7,814 (64.04)	9,303 (72.25)	0.05
No. (%) of individuals living in a household having specific place for waste disposal	12,003 (98.64)	12,675 (98.58)	0.79
No. (%) of individuals living in owned house	3,328 (27.27)	3,562 (27.68)	1.00
No. (%) of individuals living in a household owning refrigerator	2,539 (20.80)	2,705 (21.01)	0.98
No. (%) of individuals living in a household owning motorbike	914 (7.49)	747 (5.80)	0.15
No. (%) of individuals living in a household owning telephone	2,460 (20.15)	2,303 (17.88)	0.48
No. (%) of individuals living in a household owning all luxury items[4)]	601 (4.92)	520 (4.04)	0.34
No. (%) of individuals living in a household owning at least one luxury item[4)]	3,421 (28.03)	3,383 (26.27)	0.62
Mean (SD)† monthly household expenditure (Indian Rupee)	3743.71 (2,352.8)	3687.39 (2,547.4)	0.74
No. (%) of individuals having high monthly household expenditure[5)]	5,195 (43.62)	5,533 (43.34)	0.72
Mean (SD)† monthly per-capita expenditure[6)] (Indian Rupee)	621.14 (472.91)	611.56 (454.66)	0.83
No. (%) of individuals having high monthly per-capita expenditure[5)]	4,750 (39.89)	5,124 (40.14)	0.99
No. (%) of age eligible individuals for the trials living in a large cluster[7)]	5,281 (43.27)	6,864 (53.30)	0.49
Mean (SD)† household size (all individuals at *zero-time*)	7.34 (4.17)	7.20 (3.91)	0.69
Mean (SD)† distance (m) from household to the nearest health outpost	153.29 (78.57)	159.35 (77.50)	0.71
No. (%) of individuals living in household longer distance to health outpost[8)]	5,948 (48.73)	6,593 (51.20)	0.54
No. (%) of individuals living in lager cluster size of adults at *zero-time* stratification[9)]	6,978 (57.17)	6,749 (52.41)	0.50
No. (%) of individuals living in lager cluster size of paediatrics at *zero-time* stratification[10)]	7,471 (61.21)	8,709 (67.63)	0.65

* The p-values are derived by comparing the differences between the two groups adjusted for cluster effects (Using GEE with the logit link function for dichotomous variables and the identity link function for dimensional variables).

[†] Mean and Standard Deviation (SD) was adjusted for cluster effects.

[1)] Hindus compared to non-Hindus (Muslims, Christians, others)

[2)] Stable occupation: professional, service, shop owner, or farm owner

[3)] Safe toilet: use of own (non-shared) flush toilet

[4)] Luxury items: Refrigerator, motorbike, and/or washing machine

[5)] Higher (household/per-capita household) expenditure: More than the median household/per-capita household expenditure; median: median individual non-vaccinee

[6)] Per-capita household expenditure: calculated from total household expenditure divided by the number of active members in the household prior to vaccination (1USD = 45 Indian Rupees); median: median individual non-vaccinee

[7)] Larger cluster: More than the median cluster; median: median individual non-vaccinee (not median cluster)

[8)] Longer distance to the nearest health outpost: more than the median distance to the nearest health outpost; median: median individual non-vaccinee

[9)] Larger cluster size of adults at *zero-time* stratification: ≥500 adults (age>18) per cluster at stratification.

[10)] Larger cluster size of paediatrics at *zero-time* stratification: ≥200 paediatrics (age≤18) per cluster at stratification.

Table 3-10: Characteristics of the clusters at *zero time* for non-vaccinee population, Kolkata, India.

Randomized population (n=25,083) unless otherwise noted

Variables	Vi cluster (clusters=40)	Hep A cluster (clusters=40)	Total (clusters=80)	p-value[†]
Cluster stratification:				
<500 adults and <200 children	4	6	10	0.76
<500 adults and ≥200 children	13	14	27	
≥500 adults and <200 children	14	10	24	
≥500 adults and ≥200 children	9	10	19	
Mean (SD; min., max.) number of children (2-18)	255.90 (117.50; 117.00, 506.00)	273.03 (115.42; 131.00, 508.00)	264.46 (116.04; 117.00, 508.00)	0.51
Median number of children (2-18)	220.50	217.00	217.00	0.43
Mean (SD; min., max.) number of Adults (19+)	503.05 (84.47; 287.00, 694.00)	500.03 (93.16; 319.00, 734.00)	501.54 (88.37; 287.00, 734.00)	0.88
Median number of Adults (19+)	510.50	504.00	510.00	0.63
Mean (SD; min, max) mean age of persons in cluster	29.05 (5.03; 22.08, 36.21)	28.47 (4.60; 22.10, 36.07)	28.76 (4.80; 22.08, 36.21)	0.59
Median mean age of persons in cluster	28.79	27.67	28.09	0.59
Mean (SD; min., max.) number of households	142.03 (27.18; 72.00, 208.00)	145.58 (36.10; 84.00, 244.00)	143.80 (31.80; 72.00, 244.00)	0.62
Median number of households	142.50	140.00	142.00	0.81
Mean (SD; min., max.) number of total individuals	776.88 (135.53; 539.00, 1098.00)	792.03 (141.62; 550.00, 1081.00)	784.45 (137.94; 539.00, 1098.00)	0.63
Median number of total individuals	761.00	763.50	761.50	0.59
Mean (SD; min., max.) number of age eligible individuals	758.95 (128.57; 525.00, 1062.00)	773.05 (134.66; 544.00, 1031.00)	766.00 (131.01; 525.00, 1062.00)	0.63
Median number of age eligible individuals	745.00	743.50	745.00	0.63
Mean (SD; min., max.) number of dosed individuals	471.73 (102.97; 222.00, 645.00)	470.10 (104.20; 208.00, 704.00)	470.91(102.93; 208.00, 704.00)	0.94
Median number of dosed individuals	479.50	469.50	473.50	0.89
Mean (SD; min., max.) total study population density/10m^2	18.44 (17.78; 1.85, 69.38)	22.28 (20.08; 2.84, 87.49)	20.36 (18.94; 1.85, 87.49)	0.37
Median total study population density/10m^2	9.75	12.58	11.10	0.47
Mean (SD; min., max.) pre-vaccination incidence of typhoid fever	1.54 (1.40; 0.00, 6.25)	1.38 (1.38; 0.00, 4.91)	1.46 (1.39; 0.00, 6.25)	0.59
Median pre-vaccination incidence of typhoid fever	1.43	1.16	1.32	0.45
Mean (SD; min., max.) of vaccine coverage	60.98 (10.53; 37.50, 78.75)	60.09 (11.51; 21.42, 76.94)	60.54 (10.97; 21.42, 78.75)	0.72
Median of vaccine coverage	62.37	62.31	62.37	0.81

[†]The p-values are derived by comparing the differences between the two groups (chi-square test for categorical variables, t-test for mean comparison, non-parametric method (Wilcoxon rank sum test) for median comparison).

Table 3-11: Vaccine protection against typhoid for non-vaccinee population, Kolkata, India.

Randomized population (n=25,083)

Events	Frequency		Rate*	
	Vi (n=12,206)	Hep A (n=12,877)	Vi	Hep A
Typhoid cases[†]	16	31	0.1913	0.3511
Migration-out of study area	1476	1502	17.6451	17.0092
Deaths	198	209	2.3670	2.3668
Person-days[§]	8364929	8830499		
Average of Person-days	685.3129	685.7575		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.5450	0.2988	0.9938	0.0477

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.5552	0.3141	0.9813	0.0429
Larger cluster size of paediatrics at stratification[10)]	1.1012	0.5717	2.1212	0.7731
Larger cluster size of adults at stratification[9)]	1.2303	0.6496	2.3301	0.5248
Longer distance from the household to the health outpost[8)]	0.3053	0.1589	0.5867	0.0004
Age (years)	0.9321	0.9123	0.9524	0.0000

[†]Refer to footnotes of table in the previous section for description of the variables.

Table 3-12: Characteristics of the study population at zero time, Kolkata. India

Variables	Vaccine group		p-value*
	Vi (n=31,075)	Hep A (n=31,681)	
Mean (SD)† age (years)	29.05 (18.26)	28.47 (18.20)	0.59
No. (%) of males	16,680 (53.68)	17,111 (54.01)	0.82
No. (%) of Hindus[1)]	18,794 (60.48)	16,878 (53.27)	0.50
No. (%) of individuals living in a household with literate household head	22,998 (74.01)	22,072 (69.67)	0.23
No. (%) of individuals living in a household with household head having more than 5 years of schooling	16,633 (53.53)	16,642 (52.53)	0.75
No. (%) of individuals living in a household with important economic contributor having stable occupation[2)]	6,158 (19.82)	6,293 (19.87)	0.97
No. (%) of individuals living in a household using safe toilet[3)]	1,789 (5.82)	1,249 (4.00)	0.25
No. (%) of individuals living in a household using safe water source (tap at household level)	4,704 (15.14)	3,434 (10.84)	0.24
No. (%) of individuals living in a household using safe water source (tap or hand pump at household level)	4,726 (15.21)	3,475 (10.97)	0.25
No. (%) of individuals living in a household using boiled or filtered water	2,963 (9.54)	2,334 (7.37)	0.24
No. (%) of individuals living in a household always wash hands with soap and water after defecation	18,805 (60.54)	23,437 (73.98)	0.05
No. (%) of individuals living in a household having specific place for waste disposal	30,550 (98.58)	31,104 (98.28)	0.83
No. (%) of individuals living in owned house	8,483 (27.31)	8,859 (27.98)	0.99
No. (%) of individuals living in a household owning refrigerator	5,587 (17.98)	5,609 (17.70)	0.88
No. (%) of individuals living in a household owning motorbike	1,973 (6.35)	1,606 (5.07)	0.22
No. (%) of individuals living in a household owning telephone	5,165 (16.62)	4,871 (15.38)	0.58
No. (%) of individuals living in a household owning all luxury items[4)]	1,233 (3.97)	1,035 (3.27)	0.33
No. (%) of individuals living in a household owning at least one luxury item[4)]	7,552 (24.30)	7,301 (23.05)	0.64
Mean (SD) † monthly household expenditure (Indian Rupee)	3,624.65 (2,197.0)	3,524.72 (2,422.1)	0.48
No. (%) of individuals having high monthly household expenditure[5)]	12,938 (42.36)	12,990 (41.30)	0.49
Mean (SD) † monthly per-capita expenditure[6)] (Indian Rupee)	598.71 (418.27)	580.99 (409.51)	0.63
No. (%) of individuals having high monthly per-capita expenditure[5)]	12,545 (41.07)	12,760 (40.57)	0.79
No. (%) of age eligible individuals for the trials living in a large cluster[7)]	13,681 (44.03)	17,404 (54.94)	0.37
Mean (SD) † household size (all individuals at *zero-time*)	7.18 (3.99)	7.10 (3.76)	0.79
Mean (SD) † distance (m) from household to the nearest health outpost	152.73 (79.34)	158.90 (78.19)	0.71
No. (%) of individuals living in household longer distance to health outpost[8)]	14,859 (47.82)	16,516 (52.13)	0.56
No. (%) of individuals living in lager cluster size of adults at *zero-time* stratification[9)]	18,798 (60.49)	17,117 (54.03)	0.50
No. (%) of individuals living in lager cluster size of paediatrics at *zero-time* stratification[10)]	18,138 (58.37)	20,118 (63.50)	0.65

* The p-values are derived by comparing the differences between the two groups adjusted for cluster effects (Using GEE with the logit link function for dichotomous variables and the identity link function for dimensional variables).

† Mean and Standard Deviation (SD) was adjusted for cluster effects.

[1)] Hindus compared to non-Hindus (Muslims, Christians, others)

[2)] Stable occupation: professional, service, shop owner, or farm owner

[3)] Safe toilet: use of own (non-shared) flush toilet

[4)] Luxury items: Refrigerator, motorbike, and/or washing machine

[5)] Higher (household/per-capita household) expenditure: More than the median household/per-capita household expenditure; median: median individual in entire study population

[6)] Per-capita household expenditure: calculated from total household expenditure divided by the number of active members in the household prior to vaccination (1USD = 45 Indian Rupees); median: median individual in entire study population

[7)] Larger cluster: More than the median cluster; median: median individual in entire study population (not median cluster)

[8)] Longer distance to the nearest health outpost: more than the median distance to the nearest health outpost; median: median individual in entire study population

[9)] Larger cluster size of adults at *zero-time* stratification: ≥500 adults (age>18) per cluster at stratification.

[10)] Larger cluster size of paediatrics at *zero-time* stratification: ≥200 paediatrics (age≤18) per cluster at stratification.

Table 3-13: Characteristics of the clusters at *zero time* for all study population, Kolkata, India.

Randomized population (n=62,756) unless otherwise noted

Variables	Vi (clusters=40)	Hep A (clusters=40)	Total (clusters=80)	p-value[†]
Cluster stratification:				
<500 adults and <200 children	4	6	10	0.76
<500 adults and ≥200 children	13	14	27	
≥500 adults and <200 children	14	10	24	
≥500 adults and ≥200 children	9	10	19	
Mean (SD; min., max.) number of children (2-18)	255.90 (117.50; 117.00, 506.00)	273.03 (115.42; 131.00, 508.00)	264.46 (116.04; 117.00, 508.00)	0.51
Median number of children (2-18)	220.50	217.00	217.00	0.43
Mean (SD; min., max.) number of Adults (19+)	503.05 (84.47; 287.00, 694.00)	500.03 (93.16; 319.00, 734.00)	501.54 (88.37; 287.00, 734.00)	0.88
Median number of Adults (19+)	510.50	504.00	510.00	0.63
Mean (SD; min, max) mean age of persons in cluster	29.05 (5.03; 22.08, 36.21)	28.47 (4.60; 22.10, 36.07)	28.76 (4.80; 22.08, 36.21)	0.59
Median mean age of persons in cluster	28.79	27.67	28.09	0.59
Mean (SD; min., max.) number of households	142.03 (27.18; 72.00, 208.00)	145.58 (36.10; 84.00, 244.00)	143.80 (31.80; 72.00, 244.00)	0.62
Median number of households	142.50	140.00	142.00	0.81
Mean (SD; min., max.) number of total individuals	776.88 (135.53; 539.00, 1098.00)	792.03 (141.62; 550.00, 1081.00)	784.45 (137.94; 539.00, 1098.00)	0.63
Median number of total individuals	761.00	763.50	761.50	0.59
Mean (SD; min., max.) number of age eligible individuals	758.95 (128.57; 525.00, 1062.00)	773.05 (134.66; 544.00, 1031.00)	766.00 (131.01; 525.00, 1062.00)	0.63
Median number of age eligible individuals	745.00	743.50	745.00	0.63
Mean (SD; min., max.) number of dosed individuals	471.73 (102.97; 222.00, 645.00)	470.10 (104.20; 208.00, 704.00)	470.91(102.93; 208.00, 704.00)	0.94
Median number of dosed individuals	479.50	469.50	473.50	0.89
Mean (SD; min., max.) total study population density/10m^2	18.44 (17.78; 1.85, 69.38)	22.28 (20.08; 2.84, 87.49)	20.36 (18.94; 1.85, 87.49)	0.37
Median total study population density/10m^2	9.75	12.58	11.10	0.47
Mean (SD; min., max.) pre-vaccination incidence of typhoid fever	1.54 (1.40; 0.00, 6.25)	1.38 (1.38; 0.00, 4.91)	1.46 (1.39; 0.00, 6.25)	0.59
Median pre-vaccination incidence of typhoid fever	1.43	1.16	1.32	0.45
Mean (SD; min., max.) of vaccine coverage	60.98 (10.53; 37.50, 78.75)	60.09 (11.51; 21.42, 76.94)	60.54 (10.97; 21.42, 78.75)	0.72
Median of vaccine coverage	62.37	62.31	62.37	0.81

[†]The p-values are derived by comparing the differences between the two groups (chi-square test for categorical variables, t-test for mean comparison, non-parametric method (Wilcoxon rank sum test) for median comparison).

Table 3-14: Vaccine protection against typhoid for all study population, Kolkata, India.

Randomized population (n=62,756)

Events	Frequency		Rate*	
	Vi (n=31,075)	Hep A (n=31,681)	Vi	Hep A
Typhoid cases[†]	50	127	0.2307	0.5761
Migration-out of study area	2588	2587	11.9404	11.7349
Deaths	387	403	1.7855	1.8281
Person-days[§]	21674266	22045260		
Average of Person-days	697.4824	695.8511		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4005	0.2616	0.6131	0.0000

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4298	0.2937	0.6291	0.0000
Larger cluster size of paediatrics at stratification[10)]	0.7811	0.4430	1.3774	0.3933
Larger cluster size of adults at stratification[9)]	1.2311	0.8167	1.8558	0.3206
Hindus (0:others; 1:hindu)	0.6083	0.3412	1.0845	0.0920
Longer distance from the household to the health outpost[8)]	0.6552	0.4240	1.0126	0.0570
Individuals with high per-capita monthly expenditure[4)]	0.6360	0.4545	0.8900	0.0083
Age (years)	0.9125	0.8983	0.9269	0.0000

[†]Refer to footnotes of table in the previous section for description of the variables.

3.5.6 Evaluation of protection conferred by Vi polysaccharide vaccine against paratyphoid fever

To assess whether the evaluation of protection by Vi polysaccharide vaccine against typhoid fever could have been distorted by bias, due to the fact that the presentations of the Vi and hepatitis A vaccines were not identical, we analyzed Vi protection against enteric fever due to *S.* Paratyphi A. *S.* Paratyphi A was isolated from blood cultures of 54 recipients of Vi polysaccharide vaccine (.41 episodes per 100,000 person-days of follow-up) and 50 recipients of hepatitis A vaccine (.38 episodes per 100,000 person-days of follow-up). There was no suggestion of protection by Vi polysaccharide vaccine against *S.* Paratyphi A infection in either simple (PE= -7%) or adjusted (PE=-8%) analyses (Table 3-15).

Table 3-15: Vaccine protection against paratyphoid in Kolkata, India

Randomized population (n=37,673)

Events	Frequency		Rate*	
	Vi (n=18,869)	Hep A (n=18,804)	Vi	Hep A
Paratyphoid cases[†]	54	50	0.4060	0.3779
Migration-out of study area	1114	1084	8.3746	8.1921
Deaths	189	194	1.4208	1.4661
Person-days[§]	13302114	13232198		
Average of Person-days	704.9719	703.6906		

*Rate = no. of events / person-days X 100,000

[†]A paratyphoid case is defined by culture positive for *S.* Paratyphi A or B and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	1.0749	0.7160	1.6138	0.7275

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	1.0842	0.7479	1.5718	0.6696
Larger cluster size of paediatrics at stratification[10)]	0.7902	0.4621	1.3514	0.3898
Larger cluster size of adults at stratification[9)]	1.5649	1.0489	2.3348	0.0283
Hindus (0:others; 1:hindu)	0.6102	0.3665	1.0160	0.0576
Typhoid IR during 12 months before vaccination	1.1099	0.9802	1.2568	0.0999
Individuals living in a household having specific place for waste disposal	0.3332	0.1325	0.8379	0.0195
Age (years)	0.9524	0.9384	0.9665	0.0000

[†]Refer to footnotes of table in the previous section for description of the variables.

3.6 Discussion

The findings of the cluster-randomized effectiveness trial indicate that Vi polysaccharide vaccine conferred 61% protection against typhoid fever in a general population in Kolkata, India, and was associated with minimal side-effects. Importantly, high-grade vaccine protection was observed in children vaccinated before the age of five years. Also the trial demonstrated significant herd protective effects, as demonstrated by indirect protection of non-vaccinees by Vi vaccination. Before discussing the implications of these findings, it is important to consider the potential limitations of the trial.

3.6.1 Potential limitations

The major limitation of the trial was that the two vaccines were not packaged in an identical fashion. We attempted to minimize the effect of this difference by not identifying the vaccines other than by code and by not informing local research staff about how the two vaccines were packaged. However, it was possible that the identities of the vaccines could have been recognized by research staff. We therefore analyzed vaccine protection against *S.* Paratyphi A enteric fever (paratyphoid fever), a disease entity that presents with clinical features similar to typhoid fever, but should not have been prevented by Vi polysaccharide vaccine. Research staff members were unaware that paratyphoid fever was to be analyzed to evaluate the possibility of bias. As expected, Vi polysaccharide vaccine conferred no protection against paratyphoid fever, making it unlikely that bias affected the findings of the trial.

Another limitation of the trial was the manner in which we selected the geographical clusters that constituted the units of randomization. In a trial ideally designed to measure vaccine herd protection, the units of allocation should be clusters in which there is person-to-person transmission of the target pathogen, but between which there is negligible person-to-person transmission (Smith and Morrow 1996). Due to the logistical considerations of doing this trial in a densely populated urban setting, it was necessary to compromise on this requirement by randomizing adjacent geographic clusters in which there may have been substantial between-cluster transmission of typhoid. This compromise likely diluted the vaccine herd effects

measured in the trial and made the estimates of all three measures of vaccine protection – total, indirect, and overall – conservative.

3.6.2 Relation to previous studies

This study both confirms and extends the earlier findings of pre-licensure efficacy trials of Vi polysaccharide vaccine in Nepal and South Africa (Acharya, Lowe et al. 1987; Klugman, Gilbertson et al. 1987). Like the earlier trials, the evaluation found Vi polysaccharide vaccine to be conferring about 61% protection against typhoid fever during the two years of follow-up. However, neither of the previous trials evaluated clinical protection against typhoid fever in pre-school children, an age group now appreciated to be at high risk of typhoid fever, especially in urban areas of South Asia (Sinha, Sazawal et al. 1999; Brooks, Hossain et al. 2005; Ochiai, Acosta et al. 2008). This trial is the first to demonstrate that Vi polysaccharide vaccine can confer high-grade protection to this important age group. Moreover, this trial has demonstrated that mass vaccination with Vi polysaccharide vaccine can confer herd protective effects to non-vaccinated persons residing in the vicinity of vaccinated persons, an outcome never before evaluated for Vi polysaccharide vaccine.

3.6.3 Implications

As cited earlier, Vi polysaccharide vaccine has many properties that make it attractive as a vaccine for controlling typhoid fever in developing countries. While it has been documented in the past that Vi polysaccharide vaccine is protective in typhoid-endemic settings, and also is amenable to low-cost production by developing country producers, several concerns about Vi polysaccharide vaccine have impeded its introduction as a routine vaccine for poor populations in developing country settings. One concern was whether the vaccine could be feasibly delivered in mass immunization programs targeting broad age-groups in developing countries. As published elsewhere, the DOMI programme found both school-based and community-based mass immunization with Vi polysaccharide vaccine, in diverse Asian

settings, to be logistically and programmatically feasible (Yang, Acosta et al. 2005; Agtini, Ochiai et al. 2006; Khan, Ochiai et al. 2006; Thiem, Danovaro-Holliday et al. 2006).

Another concern has been that Vi polysaccharide vaccine might not be very protective in children under the age of five years, who constitute a high-risk group in many typhoid endemic populations. The trial demonstrated that Vi is highly protective in this age group. A third concern was that in past efficacy trials, Vi polysaccharide vaccine conferred moderate levels of protection to vaccinees. The trial demonstrated that in the urban Kolkata setting Vi polysaccharide vaccine not only conferred protection against typhoid fever to vaccinees, but also indirect protection to non-vaccinated neighbours of vaccinees. It was noteworthy that although Vi polysaccharide vaccine coverage was only 61%, the level of overall protection against typhoid by Vi among all members of the clusters assigned to Vi polysaccharide vaccine (58%) was nearly identical to the level of total protection against typhoid by Vi (61%), among individuals who actually received Vi polysaccharide vaccine. As noted earlier, had the geographic clusters been selected in a more idealized fashion, it is probable that even higher levels of overall protection would have been observed. This finding underscores the need to consider both direct and indirect protective effects in future deliberations about the use of Vi polysaccharide vaccine in programs in developing countries.

4 KARACHI EFFECTIVENESS TRIAL

4.1 Introduction

Typhoid fever is recognized as one of the most important childhood infections in Pakistan. Hospital records have shown that typhoid fever is the most important bacteraemic illness among febrile children requiring hospitalization and that *S.* Typhi is also the most common organism causing bacteraemia among children dying with diarrhoea (Bhutta, Punjwani et al. 1996). Estimates extrapolated from hospital-based studies indicate an incidence of culture-proven typhoid fever of approximately 227 cases per 100,000 persons, corresponding to an area of high endemicity. As an additional problem, multi-resistant drug isolates have emerged, rendering chemotherapy a costly health tool (Bhutta, Khan et al. 1994).

The trial was conducted in three settlements of Karachi, Pakistan between 2002 and 2006. The project took place in two large adjacent squatter settlements Sultanabad and Hijrat Colony, in Karachi. These settlements consist of a mixed ethnic population of almost 50, 000 from all over Pakistan. There are a total number of 8,000 households. The study site was selected due to reported high incidence of typhoid fever in prior studies conducted by Aga Khan University (AKU) and the willingness of the community to participate in typhoid fever control (Siddiqui, Rabbani et al. 2006). The study setting was a highly populous area representing the urban impoverished population structure of Pakistan. The majority of the population has access to private practicing physicians for medical care, whereas the bulk of the vaccinations are provided in the government-controlled and funded vaccination centres.

As typhoid remains a public health problem mainly for children, vaccination programme was considered only for children below the age of 16 years. Because school attendance rate was considered low in the impoverished parts of Pakistan, community-based approach was considered most applicable as a typhoid vaccination programme. The DOMI programme, therefore, planned the effectiveness trial in impoverished parts of Karachi in community-based targeted campaign approach.

4.2 Overview of the study

The study was conducted jointly by the Department of Paediatrics, Aga Khan University (AKU), Karachi, Pakistan and the International Vaccine Institute (IVI), Seoul, Korea. We used a cluster randomized controlled design for the trial. The study setting was divided into arbitrary geographic clusters using the Geographic Information system (GIS) imagery. For the first year vaccination in Hijrat and Sultanabad, the sites were divided into 60 clusters and for the second year vaccination in Bilal, the site was divided into 60 clusters; a total 120 clusters where each of the 60 clusters received the intervention or the active control. The intervention group was given Vi polysaccharide vaccine (Typherix®, GlaxoSmithKline) and the control group was given Hepatitis A vaccine (Havrix®, GlaxoSmithKline) in blinded manner. The choice of a cluster randomized design was based on the requirement for assessment of Vi polysaccharide vaccine effectiveness. Vaccine protection against culture-proven typhoid fever was monitored for two years after vaccination at both sites through the project health outposts. Vaccine safety was monitored by both active and passive surveillance. The primary outcome of the study, determined *a priori*, was total vaccine protection conferred by Vi polysaccharide vaccine over two years of follow-up, assessed by the comparative incidence rates of blood culture-proven typhoid fever in recipients of Vi versus hepatitis A vaccine.

4.3 Pre-study surveillance

The project took place in three squatter settlements in Karachi, Pakistan. Two large adjacent squatter settlements called Sultanabad and Hijrat Colony were included in the study since 2001. Public health based facilities include: four government-supported dispensaries, two primary care health centres served by the urban health programme of the Aga Khan University. The other settlement the Bilal colony, which is in the industrial area, was included in the study from 2003. The majority of the population has access to the private care sector for medical care, whereas the bulk of the vaccinations are provided in the government-controlled and funded vaccination centres.

Census

Two censuses were conducted to collect the household information at different time points. These censuses captured the *de jure* population. Timings of the census varied by site since Bilal colony was included after one year. In Hijrat and Sultanabad, the first census was carried out at the initiation of the study in December 2001. The same census was carried out in Bilal colony in March 2003. The first census had identified and registered residents with no plans to move within the next 3 years. Census teams visited each and every household in the study area and interviewed an adult member of the household. Though interviewing the head of the household was initially planned, it was not feasible as head of the household was usually out for work during the daytime when the census took place. Each household and each individual resident in the study area was assigned a unique study number and recorded in the project data management system. 101,937 persons were recorded in the catchment area by the study censuses conducted at baseline in 2001 in Hijrat Colony and Sultanabad and in 2003 in Bilal Colony. The second census was carried out in January 2003 and 2004 in Hijrat/Sultanabad and Bilal colony, respectively, to establish the sampling frame of the effectiveness trial (*vide infra*).

Surveillance

The disease surveillance system had been set up at the beginning of the project. Age groups under surveillance were selected as those judged by local officials to be the most appropriate targets for typhoid vaccination: 2-16 year olds.

Three project healthcare facilities were established (one in each site) and served as clinic and referral point for the patients. Passive surveillance was augmented through community health workers visiting each household weekly to encourage febrile patients to visit participating healthcare facilities, and private practitioners were encouraged to refer patients for free typhoid diagnosis and treatment at these centres. Patients residing in the catchment areas and presenting to a treatment facility with fever of 3 days or more were eligible to participate in the study. Surveillance included patients treated as outpatients as well as inpatients. Surveillance included both systematic collection of clinical information, recorded on

standardized forms, and collection of a single blood culture by venipuncture. Approximately 5 to 8 ml of blood was collected to inoculate Bactec Plus Aerobic® culture bottles (Becton Dickinson, New Jersey, USA); for younger children, about 3 to 5 ml of blood was inoculated into Bactec Peds Plus® culture bottles (Becton Dickinson, New Jersey, USA). Details on laboratory procedures are noted in section 4.4.7.

A crucial feature of the surveillance was accurate identification of all patients attending the treatment facilities to determine the study participants. To accomplish this, a computer-based identity search programme was developed using the project census data and equipped in all 3 study treatment centres. The computer-based identity search programme allowed active identity search while the patient was attended by the treating physician. This identification process was conducted by the project health personnel from the community. Case report forms from private sectors without active identity confirmation were checked with the same programme at the data management unit retrospectively. Additionally, the culture positive cases were followed up to assess disability at days 7, 14, 30, and 60. At the time of the follow up on day 7, study personnel were to confirm the identity of the individual so that no misclassification was to occur in the analyses.

Analysis

A 12-month period from each site was taken from the pre-vaccination phase to determine the annual incidence rate, rather than annualizing the whole period. The period, from August 2002 for Hijrat and Sultanabad colonies and from August 2003 for Bilal Colony, were chosen because that time the surveillance system was functioning optimally. A patient from the target population presenting to a participating treatment centre with a history of fever lasting ≥3 days was considered as having a febrile episode. A typhoid episode was defined as an episode of febrile illness during which *S.* Typhi was isolated from a blood culture. Census and surveillance data were double-entered into a custom data entry program (FoxPro, Microsoft, Washington, USA) with error and consistency check programs. Typhoid patients and not "episodes" were considered in the analysis; thus, each individual could be included only once in the numerator for calculation of incidence. We assumed that each person residing in the study area at the time of the census contributed 12 months to the denominator. Incidence per

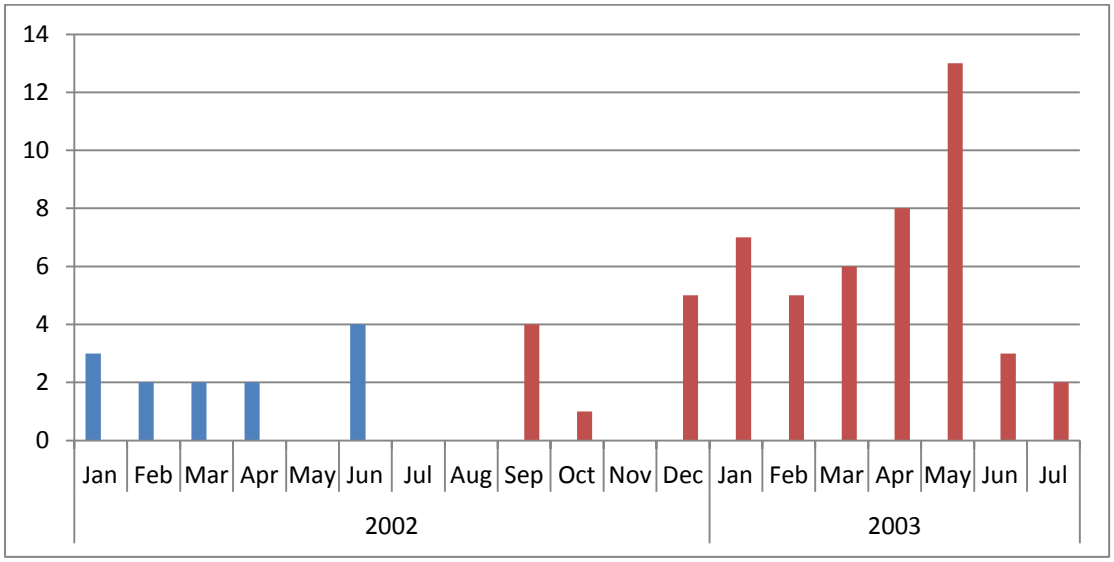
100,000 person-years was calculated using age-specific denominators from the baseline census. The numerator was the number of age-specific disease episodes. To estimate the prevalence of the disease, we considered the number of episodes per all febrile episodes registered. We calculated 95% confidence intervals for incidence using the Wilson score method. Categorical variables were compared using the χ^2 test and dimensional variables were compared using analysis of variance. A value of $P<0.05$ (two-tailed) was considered statistically significant. Stata/SE 8.0 (Stata Corporation, Texas, USA) was used for the analysis.

Results

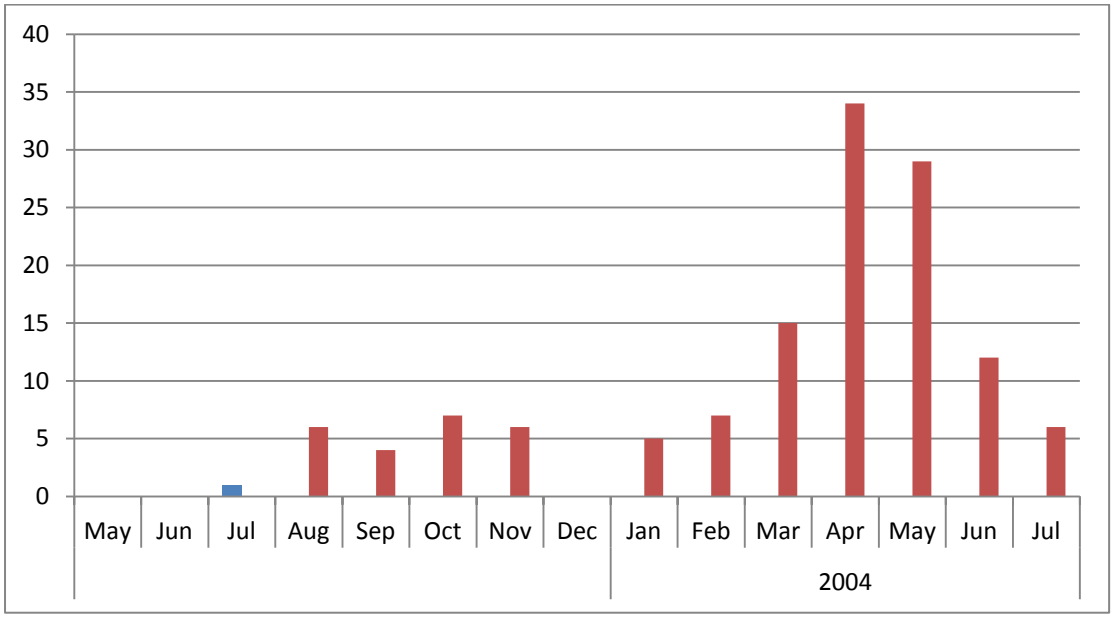
There were 7,736 episodes of fever in children and adolescents aged between 2 and 16 years from the three urban slums through the surveillance system during the preparation phase (1/Aug/2002 to 31/Jul/2003 for Hijrat Colony and Sultanabad; 1/Aug/2003 to 31/Jul/2004 for Bilal Colony), of which, 7,415 episodes had blood samples taken. 189 samples yielded *S.* Typhi (Figure 4-1). The incidence rate of *S.* Typhi was 451.7/100,000 persons per year, where the rate was highest in the age group 2 to 5 years (573.2/100,000 persons per year), followed by the age group 5 to 16 years (412.9/100,000 persons per year). Prevalence of the disease was almost the same for the two age groups 25.3 cases per 1,000 febrile episodes per year in 2 to 5 years and 25.6 cases per 1,000 febrile episodes per year in 5 to 16 years. 127 of 189 *S.* Typhi isolates were subjected for all antimicrobial agent susceptibility tests. Nearly 60% of the 127 isolates were resistant to chloramphenicol, ampicillin, TMP-SMX, and nalidixic acid. Multidrug resistance (resistant to 3 first-line antimicrobials agent: chloramphenicol, ampicillin and TMP-SMX) was observed from 85/127 (67%) isolates and nalidixic acid resistance was found in 75/127 (59%) isolates. None of the *S.* Typhi isolates was resistant to ciprofloxacin or to third-generation cephalosporins (cephalothin).

Figure 4-1: Blood culture confirmed S. Typhi cases from Karachi, Pakistan during pre-vaccination surveillance period

Sultanabad and Hijrat colonies



Bilal Colony



Red bars denote the typhoid cases from the period used to assess the typhoid disease burden. The periods from August 2002 in Sultanabad and Hijrat colonies and from August 2003 in Bilal Colony were taken after ensuring the surveillance was optimal.

4.4 Methods

4.4.1 Baseline census and definition of clusters

Two *de jure* household censuses were conducted in the study settings prior to the vaccination. Timings of the census varied by site since Bilal colony was included after one year. In Hijrat and Sultanabad colonies, the first census was carried out at the initiation of the study in December 2001. The same census was carried out in Bilal colony in March 2003. The first census had identified and registered residents with no plans to move within the next 3 years, and collected information on all household members, health seeking behaviour and willingness to participate in a future typhoid vaccine trial (*vide ante*). Each household and each individual resident in the study area was assigned a unique study number and recorded in the project data management system. The second census, or baseline census, was carried out in January 2003 and 2004 in Hijrat/Sultanabad and Bilal colony, respectively, just prior to the vaccination to define the sampling frame for the vaccination, enumerating not only the demographic information of the residents from the first census, but also the new residents fulfilling the registration criteria. The census also used Geographic Positioning System (GPS) tools to record the geographical coordinates of each household (Ali, Rasool et al. 2004). The census and geographical mapping was then used to define the 120 contiguous geographical clusters that served as the units of randomization (Figure 4-2). The populations of the clusters ranged from 519 to 1,936 and the target population per cluster ranged from 175 to 735.

Figure 4-2: Map of the study areas showing the project health centres and vaccine assignment for the 120 clusters for the Vi Effectiveness Trial in Karachi, Pakistan

These 120 clusters were randomized to receive Vi polysaccharide (green) or inactivated Hepatitis A vaccine (blue).



4.4.2 Compared Agents

Vi polysaccharide vaccine (Typherix®, GlaxoSmithKline) was the principal vaccine under study. Each vial contained 25ug of Vi polysaccharide in a single-dose suspension, administered by intramuscular injection in the deltoid region. The control vaccine was inactivated hepatitis A vaccine (Havrix®, GlaxoSmithKline). This vaccine contained 720IU inactivated hepatitis A virus in a single-dose suspension, also administered by intramuscular injection in the deltoid region. These vaccines were donated by GlaxoSmithKline. Both agents had identical presentations, in single-dose syringes, to ensure blinding. To further decrease the likelihood that these different presentations would create a bias, the compared agents were provided with identical labels giving only a code letter. Two code letters were used. Each vaccination centre provided one type of vaccine, thus participants were not exposed to the two different presentations. Members of the project involved with vaccine administration and surveillance were not informed of the identities of the codes. Additionally, vaccination teams administering the vaccines were assigned only to administer only one of the vaccines throughout the immunization campaign (*vide infra*).

4.4.3 Randomization

Assignment of the clusters to the two vaccines was stratum-randomized and blocked to ensure balanced numbers of clusters within each stratum. The baseline census provided information on distribution of characteristics on different variables for use in the randomization of clusters to Vi polysaccharide vaccine or to the control Hepatitis A group. Stratified allocation based on the size of cluster (Sultanabad/Hijrat: ≤350 versus >350 persons; Bilal: ≤450 versus >450 persons) and being in one of three study colonies was done, creating 4 strata in Sultanabad/Hijrat and 2 strata in Bilal. A statistician who was not involved directly in the implementation of the study randomized the cluster to receive one code or another, blinded to the allocation of code to the Vi polysaccharide vaccine or Hepatitis A vaccine (Figure 4-2).

4.4.4 Vaccination

The vaccines were delivered to the target population in a campaign fashion spanning one month of extensive field work. The vaccination campaign was conducted in Hijrat and Sultanabad colonies between August 12 and September 12, 2003 and in Bilal colony from July 24 to Aug 15, 2004. Children between the ages of two and sixteen years were targeted for the vaccination. Vi polysaccharide vaccine does not confer immune response to younger children, and the incidence estimates of typhoid in older than sixteen years population in Pakistan and in other parts of South Asia are lower compared to younger children. Therefore we did not include older than 16 years age population in the study. All children, residing in the study setting, included in the household census data base conducted by the project and showed up at the time of vaccination, were eligible. Married or female children older than twelve years were not included due to the absence of safety results for use of Vi polysaccharide vaccine in pregnant women. The vaccination staff used the study identification cards for identification of eligible children. The identification cards were distributed by field teams to the household heads prior to the start of the vaccination campaign. The vaccination teams were headed by trained physicians and had trained vaccinators. The vaccination team head ensured proper identification, proper administration of the vaccine and proper recording of the information. These targeted children were assessed for contraindication (acute fever ≤37.5 °C at the time of visit) and informed consent prior to vaccination.

Each administered dose of vaccine was entered into pre-printed vaccine record books, listing each resident by household. All vaccinees were observed for 30 minutes following dosing to detect and manage immediate adverse events. Vaccination was supervised by a supervisor assigned to two or three teams, and external observers monitored and documented several features (consent process, injection safety, data recording, and cold chain) on designated forms. Vaccination was undertaken in special vaccination centres, one for each cluster, and the schedule of vaccination was arranged so that vaccination with Vi polysaccharide vaccine and Hepatitis A vaccine was temporally balanced.

Prior to the vaccine campaign, social mobilization was done through the Imams of the local mosques, private physicians from the area, and local influential persons. Additionally, local

social scientists organized interactive sessions to inform community members about the trial. The day before the vaccination of a cluster, cluster households were visited to provide information about the trial and were given household ID cards that included the site, date, and time for vaccination. In addition, individuals visiting vaccination posts on the day of immunization were given information about the nature of the trial, expected risks and benefits, and procedural details as part of the informed consent. Upon agreeing to participate, a thumbprint or signature was affixed on the vaccination record book together with the signature of a witness.

After the conclusion of surveillance, age-eligible residents of clusters originally assigned to Vi polysaccharide vaccine were offered a dose of Hepatitis A vaccine, and age-eligible persons originally assigned to Hepatitis A vaccine were offered a dose of Vi polysaccharide vaccine.

4.4.5 Surveillance for adverse events

Adverse events following immunization was recorded at various levels. The vaccinees were requested to stay in the vaccination centres at least for 30 minutes after the vaccine administration for assessment of immediate adverse events. The vaccination teams were trained and equipped to manage any untoward reaction following immunization based on WHO serious adverse event management and reporting guidelines (World Health Organization 1997). The vaccinees or their parents were advised to consult the physician of the vaccination centres or visit the study health centres if the child had fever or any other health related problem following vaccination. A non-solicited adverse event form was filled in for children presenting to health centres with fever, pain or other associated symptoms after vaccination. A sub-sample of 480 vaccinees, four from each cluster, was selected to be visited daily for 3 days after vaccination for the assessment of adverse event and a solicited adverse event form was filled in. Among them, 242 vaccinees agreed to be followed up and we did not replace those who did not take the vaccine or did not consent to provide the information (n=238). At each visit, all adverse events were observed by the trained study personnel or reported by the parents/guardians of the subject and were recorded. Additionally,

passive surveillance was implemented for all patients from the study area who presented for care at the study health centres (*vide infra*).

All deaths occurring in the study population during this same period were monitored by project community health workers who visited each household weekly, and verbal autopsies were conducted. All adverse events following immunization, deaths, and verbal autopsies, all of them causal or not causal, were entered onto special study data forms by physicians, which also included physician assessments as to whether the noted adverse event could have been causally related to one the vaccines tested in trial. Serious adverse events, defined as in ICH GCP guideline (International Conference on Harmonization 1996), were monitored by a local clinical monitor; the Data and Safety Monitoring Board; the study secretariat at the International Vaccine Institute; and the manufacturer.

4.4.6 Surveillance for typhoid fever

The study population was followed for 2 years after vaccination. Identification of *Salmonella* infection was done through three study health centres specifically established for the study. These health centres gave routine care for all outpatient visits, and referred patients with severe disease for hospital care. The health centres were staffed with physicians trained in paediatrics in major public teaching hospitals, and local staff who assisted the physician in blood collection, identification through the study data base, and follow up visits. The study health centres operated from 10:00AM to 10:00PM in two shifts and provided consultation to children with fever and other common illnesses. A child, who presented with a history of fever for three or more days, was included in the surveillance and venous blood drawn for microbiological assessment of typhoid fever. For the blood culture, approximately 5 to 8 ml of blood was collected to inoculate Bactec Plus F/ Aerobic® culture bottles (Becton Dickinson, New Jersey, USA); for younger children, about 3 to 5 ml of blood was inoculated into Bactec Peds Plus® culture bottles (Becton Dickinson, New Jersey, USA).

We developed a special mechanism for referral procedures for the private doctors seeing eligible children that fulfilled the case definition of suspected typhoid fever (Khan, Sahito et al.

2006). The physicians recorded information on history, clinical presentation and household practices on standardized data collection instruments for eligible children. The febrile children were initially treated with antipyretics if the focus of fever was not identified. In case the study physicians after complete physical examination considered starting antibiotics, first line antibiotics for *Salmonella* were used. At the follow up visit when the culture results were available, the study physicians assessed the child again and if needed modified the treatment. The study team also visited the homes of the children for whom the blood culture showed growth for *S.* Typhi. The home visit aimed for assessment of the clinical outcome and confirmation of residence of the cases. Each positive case of *S.* Typhi was followed for up to one year for any long term sequelae.

Several methods were used to help identify subjects seen in the surveillance. Subjects were encouraged to bring study identification cards issued during the census or during vaccination when presenting for care. In addition, in all of the treatment sites a computerized version of the most recent census of the population was used to identify each subject, assisted by a special search algorithm developed for the study. These data sources allowed health centre staff to locate the subject's census number for later linkage with the vaccination database at the conclusion of the study. The vaccine code received by subjects was kept away from the AKU to discourage any tabulation of cases by code letter. Finally, whenever a blood culture yielded either *S.* Typhi or *S.* Paratyphi, a study team was dispatched to the home of the patient within seven days to verify that the subject whose name was given at the treatment site had indeed visited the treatment site for care on the date noted in the surveillance.

4.4.7 Laboratory procedures

Laboratories for microbiological evaluations of blood cultures collected by the project were set up at each site. Approximately 5 to 8 ml of blood was collected and used to inoculate Bactec Plus Aerobic® culture bottles (Becton Dickinson, New Jersey, USA); for children under the age of 5 years, 3 to 5 ml of blood was inoculated into Bactec Peds Plus® culture bottles (Becton Dickinson, New Jersey, USA). Blood culture bottles were transported to the

laboratory the same day of the sampling, incubated, and observed for growth. When bacterial growth was confirmed, the blood culture bottle was sub-cultured on MacConkey agar plate. In the absence of visible growth, the bottle was sub-cultured on MacConkey agar on days 1, 2, 4 and 7. The bottles were incubated for a minimum of 10 days before discarding. Lactose non-fermenting colonies from MacConkey agar were screened using Kligler's iron agar (KIA). The colonies suggestive of S. Typhi (acid butt, alkaline slant, trace amount of hydrogen sulfide) were subjected to slide agglutination with Vi antiserum, Salmonella D1 group-specific antiserum, Factor 9 O antiserum and factor d H antiserum as appropriate (Bopp, Brenner et al. 1999). All presumptive S. Typhi isolates were sent to a reference laboratory (Wellcome Trust Research Unit, Ho Chi Minh City, Vietnam) for confirmation. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method on Muller-Hinton agar with standard antimicrobial discs (Becton Dickenson Laboratories, New Jersey, USA). Resistance to five antimicrobial agents, chloramphenicol, ampicillin, TMP-SMX (trimethoprim-sulphamethoxazole), nalidixic acid, and ceftriaxone was tested routinely. Resistance to ciprofloxacin was tested by minimum inhibitory concentration (MIC) method only when nalidixic acid resistance was observed in disc diffusion method.

Consultants from AKU visited frequently the field site laboratories to assess the quality and correctness of blood collection techniques and ensure proper transportation to the AKU laboratories. The AKU laboratory runs on laboratory guidelines of international standards (Certified by ISO and Joint Commission International Accreditation (JCIA)).

4.4.8 Surveillance for demographic events

Following vaccination, closing census was carried out in October 2005 and September 2006 in Hijrat/Sultanabad and Bilal colony, respectively, in the same manner as the second census. During the census, information was obtained on all out-migrations, internal migrations, and deaths among the population present at the time of vaccination. In addition, deaths identified by the community health workers were cross checked with the census information.

4.4.9 Definitions

Several definitions, formulated *a priori*, were required for the analysis of data from the trial. A *household* was defined as all persons residing together and sharing the same cooking pot. *Zero time*, denoting the time marking the onset of follow-up, was the date of vaccination for recipients of Vi or hepatitis A vaccines, and, for non-vaccinees (i.e. those whose parent/guardian did not consent to receive the vaccine, medically ineligible to receive the vaccine on the vaccination day, or absent on the vaccination day), was the date at which 50% of vaccinees in the same cluster had received a vaccine. Since there were patients seeking care multiple times in a short period of time, defining an episode was considered important so that those visits were concatenated into one illness period. A *febrile episode* comprised treatment visits of patients with subjective or objective (≥38 °C axillary) fever in which the recalled date of onset for fever for one visit was within 14 days of presentation for the next such visit. An episode of *typhoid fever* was a febrile episode in which *S.* Typhi was isolated from at least one blood culture. An episode of *paratyphoid fever* was a febrile episode in which *S.* Paratyphi was isolated from at least one blood culture, but *S.* Typhi was not isolated from any blood cultures. The *onset* of these episodes was taken as the recalled onset date of fever by the patient for her/his first treatment visit of the episode.

4.4.10 Analytic strategies

A detailed analytical plan based on the study protocol was composed prior to conducting any analysis. The analytical plan and associated statistical program was made based on re-randomized data, completely independent of the assigned randomization codes to ensure maintaining the blinding. This analytical plan was submitted to the Data and Safety Monitoring Board for approval. Analytical plan is included as an appendix.

In short, statistical comparison of the two groups for baseline individual-level variables employed Generalized Estimating Equations (GEE) to adjust for membership in the randomized clusters, with the logit link function used for dichotomous variables and the identity link function for dimensional variables (Liang and Zeger 1986). Cluster-level baseline

variables were compared using the Student t test for dimensional variables and the chi square test for categorical variables. Evaluation of vaccine safety entailed comparison of recipients of Vi polysaccharide vaccine with those of hepatitis A vaccine for immediate adverse events, for events ascertained daily for three days after dosing in a subset of participants, for medical events detected in study clinics and hospitals during the month after dosing, and for mortality, including cause-specific mortality, during the month following dosing. Statistical evaluation of these outcomes, which were dichotomous, employed GEE with the logit link function to adjust for cluster allocation (Liang and Zeger 1986).

Vaccine protection was assessed in several ways. In each approach, enteric fever episode outcomes were analyzed if their onset occurred from the day after zero time, and persons were analyzed according to the vaccine assigned to their clusters. The primary objective of the study was to evaluate *total vaccine protection*, comparing rates of episodes of typhoid fever in recipients of Vi polysaccharide vaccine versus recipients of hepatitis A vaccine. As secondary objectives we evaluated two additional types of vaccine protection. We estimated the *indirect vaccine protection* conferred by Vi polysaccharide vaccine against typhoid fever by comparing the rates of typhoid fever in non-vaccinees in clusters assigned to Vi polysaccharide vaccine versus those for non-vaccinees in the clusters assigned to hepatitis A vaccine. We evaluated *overall vaccine protection* by comparing rates of typhoid fever in all persons residing in clusters assigned to Vi polysaccharide vaccine versus all persons residing in clusters assigned to hepatitis A vaccine (Halloran, Struchiner et al. 1997). Finally, to assess whether bias could have distorted the rates of typhoid fever in the Vi polysaccharide vaccine versus the hepatitis A vaccine clusters, we evaluated total protection by Vi polysaccharide vaccine against episodes of paratyphoid fever (all of which were due to *S.* Paratyphi A in the study), a disease for which protection by Vi polysaccharide vaccine was not anticipated but which has clinical characteristics closely resembling typhoid fever. In all analyses, we evaluated vaccine protection against first episodes of the disease outcome during surveillance after zero time.

To estimate vaccine protection, we used Cox proportional hazard regression models, verifying first that the proportionality assumption was satisfied for all independent variables (Lin, Wei et

al. 1993). These models compared the time from zero time to the first episode of the analyzed outcome in members of the Vi polysaccharide vaccine group versus members of the hepatitis A vaccine group; censoring events for these analyses included migration out of the zero-time cluster, death, or completion of the two-year, post-zero time follow-up, whichever came first. These were ascertained through censuses conducted during the follow up period. Relative rates (RRs) of the target outcome in the Vi versus the hepatitis A groups were estimated by exponentiating the coefficient for the vaccine variable in these models, and vaccine protective effectiveness (PE) was estimated as (1- RR) X 100%. Standard errors for the coefficients for the vaccine variable in the models were used to estimate P values and 95% confidence intervals for the RRs. Simple analyses of vaccine impact were adjusted for the design effect of the cluster randomization in these models with robust sandwich variance estimates, also known as Huber-White standard error estimates (Reid and Crèpeau 1985; Lin and Wei 1989). Final adjusted estimates were obtained from models that also included the variables used for stratified randomization, as well as potentially confounding variables independently associated with the time to the event at $P<0.1$ in a backward selection algorithm. Finally, to evaluate heterogeneity of vaccine protection among different subgroups of participants, we evaluated interaction terms between the vaccination and subgroup variables in these models. All P values and 95% confidence intervals for these analyses were estimated in a two-tailed fashion.

4.4.11 Sample size requirement

The unit of randomization for the study was a geographic cluster. A total of 120 clusters inhabiting approximately 27,181 children were required to have 80% power to detect a 50% vaccine protection at 5% level of significance. The sample size calculation using the Hayes and Bennett formula assumed a minimum cumulative typhoid incidence of 2.8 per 1,000 persons during 2 years. The sample was adjusted for a 10% attrition rate in two years (Hayes and Bennett 1999).

4.4.12 Monitoring and ethics

A Data and Safety Monitoring Board specially constituted for the trial approved the study protocol, monitored serious adverse events during the study, and verified that the dataset was frozen and the analytic plan was finalized prior to analyses of the data. The Institutional Review Board at the International Vaccine Institute, and the Ethical Review Committee of the Aga Khan University approved the protocol and monitored the progress of the study. Information regarding the study was provided first to the community influential persons (i.e. community leaders, imams, and general practitioners) for their consent, followed by community information sessions. During the time of the vaccination, informed consent was obtained from parents or guardians of all participants and recorded in the vaccination record book in a form of written signature or thumbprint (see appendix for vaccination procedure and information provided to obtain consents).

No agreements were signed regarding confidentiality of the data among donors, the study sponsor (the International Vaccine Institute), the vaccine manufacturer, and the investigators. The vaccine manufacturer donated the vaccines and conducted serologic assays in a blinded manner but did not participate in the study design or statistical analysis.

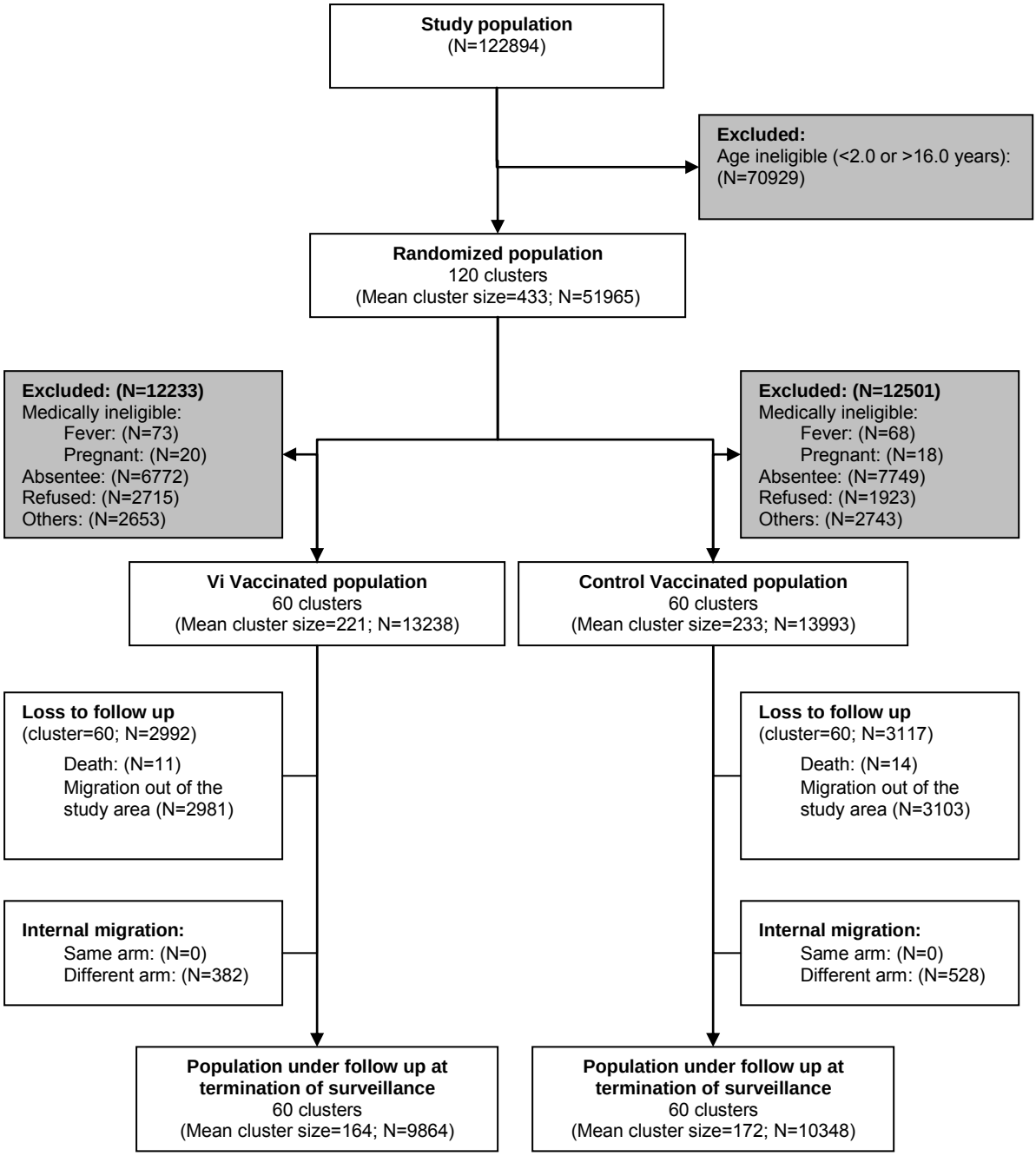
4.5 Results

4.5.1 Assembly of the study population

The total population of the three study colonies (Sultanabad, Bilal and Hijrat) at *zero time* was 122,894 and 51,965 (42%) were children in the target age group of 2 to 16 years (Figure 4-3). A total of 27,231 individuals in 120 clusters, with a mean cluster size of 433 eligible children, were randomized to either receive Vi polysaccharide vaccine (13,228) or the control Hepatitis A vaccine (13,993). During two years of follow up, 6,134 children were lost to follow up: deaths (25) and migration out (6,109). An additional 910 (3%) children were not included in

the final analysis due to migration to another cluster, different from their cluster of randomization.

Figure 4-3: CONSORT chart of the study population of the Karachi Vi Effectiveness Trial



***Definitions of exclusions:**
Fever – acute febrile (axilliary ≥38ºC) persons on the day of vaccination
Pregnant – persons being pregnant on the day of vaccination

4.5.2 Comparability of the compared groups at zero time

The study participants were similar in a wide variety of socio-demographic and economic characteristics at baseline. Table 4-1 and Table 4-2 compare vaccinated individuals in the 60 Vi polysaccharide vaccine clusters with those in the 60 Hepatitis A vaccine clusters. All dosed subjects received the vaccine assigned to the clusters. The mean vaccination coverage in the Vi polysaccharide vaccine group was 52% and in the Hepatitis A group was 53%.

Table 4-1: Characteristics of individuals at zero time, Karachi, Pakistan

Variables	Vaccine group		p-value*
	Vi (n=13,238)	Hep A (n=13,993)	
Individual Level			
Mean (SD)† of age (years)	8.35 (3.80)	8.42 (3.80)	0.36
No. (%) of children aged less than 5 years	3,154 (23.83)	3,324 (23.75)	0.85
No. (%) of male	6,899 (52.12)	7,191 (51.39)	0.26
Household Level			
No. (%) of individuals living in a household with household head having any education[1)]	5,405 (40.83)	6,043 (43.19)	0.21
No. (%) of individuals living in a household using safe toilet[2)]	10,933 (82.69)	11,952 (85.49)	0.26
No. (%) of individuals living in a household using safe water source[3)]	5,340 (40.39)	5,195 (37.14)	0.71
No. (%) of individuals living in owned house	8,898 (67.25)	9,102 (65.12)	0.15
No. (%) of individuals living in a household owning refrigerator	3,445 (26.02)	3,853 (27.54)	0.67
No. (%) of individuals living in a household owning motorbike	810 (6.12)	845 (6.04)	0.96
No. (%) of individuals living in a household owning washing machine	5,936 (44.84)	6,779 (48.45)	0.33
No. (%) of individuals living in a household owning at least one luxury items[4)]	6,579 (49.70)	7,432 (53.11)	0.35
Mean (SD) † monthly household income (PKR)	5682.97 (4692.3)	5802.53 (4428.0)	0.58
No. (%) of individuals with high monthly household income[5)]	4,649 (36.97)	4,907 (38.33)	0.64
Mean (SD) † per-capita monthly income[6)] of the household (PKR)	710.93 (571.49)	731.17 (818.86)	0.38
No. (%) of individuals with high per-capita monthly income[5)] of the household	5,909 (46.99)	6,320 (49.37)	0.21
Mean (SD) † total individuals in the household	9.05 (4.66)	9.11 (4.78)	0.78
Mean (SD) † distance (m) from household to the project health centre	401.45 (6458.5)	373.79 (5985.6)	0.46
No. (%) of individuals living in household longer distance from the household to the project health centre[7)]	7,167 (54.14)	6,447 (46.07)	0.30
Cluster Level			
Mean (SD) number of households in the cluster	162.75 (41.37)	166.84 (39.85)	0.46
Mean (SD) number of total individuals in the cluster	1062.25 (277.05)	1107.92 (285.36)	0.33
Mean (SD) number of age eligible individuals in the cluster	452.56 (120.08)	469.03 (122.38)	0.42
Mean (SD) number of vaccinees in the cluster	240.56 (71.42)	252.79 (74.26)	0.30
Mean (SD) cluster population density /100m^2 (total individuals in the cluster/cluster area)	8.88 (3.57)	9.50 (4.41)	0.56
No. (%) of individuals living in higher cluster population density[8)]/100m^2	7,124 (53.81)	6,352 (45.39)	0.27
Mean (SD) cluster IR[9)] during 12 months before vaccination	2.33 (2.70)	2.88 (3.08)	0.24
No. (%) of individuals living in higher cluster IR during 12 months before vaccination[10)]	6,033 (45.57)	7,505 (53.63)	0.36
No. (%) of individuals living in large cluster specified in the stratification[11)]	7,582 (57.27)	8,424 (60.20)	0.72
Colony Level			
No. (%) of individuals living in Sultanabad Colony	2,672 (20.18)	2,997 (21.42)	1.00

Variables	Vaccine group		p-value*
	Vi (n=13,238)	Hep A (n=13,993)	
No. (%) of individuals living in Hijrat Colony	3,633 (27.44)	3,524 (25.18)	1.00
No. (%) of individuals living in Bilal Colony	6,933 (52.37)	7,472 (53.40)	1.00

* The p-values are derived by comparing the differences between the two groups adjusted for cluster effects (Using GEE with the logit link function for dichotomous variables and the identity link function for dimensional variables).

† Mean and standard deviation (SD) was adjusted for cluster effects.

1) Any education: any type of education is counted; compared with "no education"

2) Safe toilet: use of own (non-shared) flush toilet

3) Safe water source: consumption of tap (communal/personal) water or bottled water

4) Luxury items: Refrigerator, motorbike, and/or washing machine

5) High (household/per-capita household) income: More than the median household/per-capita household income; median derived using the vaccinated population [0: low income; 1: high income]

6) Per-capita household income: calculated from total household income divided by the number of active members in the household prior to vaccination (1USD = 60 Pakistan Rupees)

7) Longer distance to the project health centre: more than the median distance to the project health centre; median derived using the vaccinated population [0: close to the project health centre; 1: long distance to the project health centre]

8) Higher population density: more than the median cluster population density; median derived using the vaccinated population [0: low cluster population density; 1: high cluster population density]

9) Cluster IR: incidence rate calculated from the number of cases between 366 days to 1 day prior to the 1st day of vaccination in each site (1: Sultanabad and Hijrat, 2: Bilal Colony) divided by the number of total population at ZT

10) Higher cluster incidence rate during 12 months before vaccination: more than the median incidence rate during 12 months before vaccination [0: low cluster IR; 1: high cluster IR]

11) Larger/Small cluster specified in stratification:

Sultanabad/Hijrat:	Large: >350	Small: ≤350
Bilal	Large: >450	Small: ≤450

Table 4-2: Characteristics of the clusters at *zero time*, Karachi, Pakistan.

Randomized population (n=27,231) unless otherwise noted

Variables[†]	Vi (clusters=60)	Hep A (clusters=60)	Total (clusters=120)	p-value*
Mean (SD; min., max.) number of households in the cluster	153.82 (37.79; 91.00, 301.00)	158.97 (39.07; 74.00, 253.00)	156.39 (38.36; 74.00, 301.00)	0.46
Median number of households in the cluster	151.00	156.50	153.50	0.40
Mean (SD; min., max.) number of total individuals in the cluster	1000.85 (259.17; 531.00, 1936.00)	1047.38 (273.55; 519.00, 1836.00)	1024.12 (266.36; 519.00, 1936.00)	0.34
Median number of total individuals in the cluster	989.00	1016.50	998.00	0.34
Mean (SD; min., max.) number of age eligible individuals in the cluster	424.52 (118.55; 175.00, 728.00)	441.57 (117.27; 199.00, 735.00)	433.04 (117.73; 175.00, 735.00)	0.43
Median number of age eligible individuals in the cluster	410.50	434.50	419.50	0.50
Mean (SD; min., max.) number of vaccinees in the cluster	220.63 (66.87; 92.00, 458.00)	233.22 (68.13; 117.00, 459.00)	226.93 (67.51; 92.00, 459.00)	0.31
Median number of vaccinees in the cluster	214.50	222.50	219.50	0.28
Mean (SD; min., max.) cluster population density/100m^2	8.98 (3.62; 2.85, 19.06)	9.41 (4.44; 3.52, 19.63)	9.19 (4.04; 2.85, 19.63)	0.56
Median cluster population density/100m^2	8.34	7.81	8.13	0.91
Mean (SD; min., max.) cluster IR[9)] during 12 months before vaccination	2.27 (2.73; 0.00, 8.99)	2.90 (3.16; 0.00, 11.09)	2.59 (2.95; 0.00, 11.09)	0.24
Median cluster IR[9)] during 12 months before vaccination	1.64	2.25	2.01	0.29
Mean (SD; min., max.) cluster vaccine coverage[12)]	52.64 (9.55; 30.70, 68.44)	53.35 (9.44; 35.53, 79.21)	52.99 (9.46; 30.70, 79.21)	0.68
Median cluster vaccine coverage[12)]	52.82	52.41	52.48	0.98
Number of small clusters[11)]	31	29	60	0.72
Number of large clusters[11)]	29	31	60	.

*The p-values are derived by comparing the differences between the two groups (t-test for mean comparison, chi-square test for categorical variable, and non-parametric method (Wilcoxon rank sum test) for median comparison).

[†]Refer to footnotes of table in the previous section for description of the variables.

[12)] Cluster vaccine coverage was derived from population aged 2.0 to 16.0 years

4.5.3 Evaluation of adverse events following vaccination

During the 30-minute period of observation after injection with the assigned vaccine, 10 events (5 each from the Vi polysaccharide vaccine group and the hepatitis A vaccine group) were noted. All of these reported immediate cases (vomiting and pain) were followed up for a longer period and none were considered serious. Furthermore, 242 subjects were followed up for three consecutive days after dosing; 125 in the Vi polysaccharide vaccine group and 117 in the hepatitis A vaccine group to assess adverse event in solicited manner (Table 4-3). Over the entire three-day period, there were more reports of pain at injection site (5 vs. 1) and fever (4 vs. 1) from the Vi group, but none were statistically significant. No signs or symptoms were more common in the hepatitis A group than in the Vi group.

During the 30 days following dosing, 2 deaths (1 in the hepatitis A vaccine group and 1 in the Vi polysaccharide vaccine group) and one hospitalization due to injection site abscess were reported as serious adverse event among recipients of either vaccine. Neither of the death cases was associated with either of the vaccine, and for the hospitalized case, the patient recovered fully in 4 days.

Table 4-3: Distribution of solicited adverse event symptoms by vaccine received

The p-values were derived by comparing the difference between two groups using chi-square test for categorical variables, except when data are sparse Fisher's exact test was used, and t-test for continuous variable.

	Day 1			Day 2			Day 3			Any day		
	Vi (n=125)	Hep A (n=117)	P-value	Vi (n=125)	Hep A (n=117)	P-value	Vi (n=125)	Hep A (n=117)	P-value	Vi (n=125)	Hep A (n=117)	P-value
Redness	0	0	-	0	0	-	0	0	-	0	0	-
Swelling	0	0	-	0	0	-	0	0	-	0	0	-
Pain	4	1	0.371	2	1	0.498	5	1	0.214	5	1	0.214
Fever	1	0	1.000	2	1	1	4	1	0.371	4	1	0.371
Fatigue	0	1	0.483	0	0	-	1	1	1.000	1	1	1.000
headache	1	1	1.000	2	1	1.000	2	2	1.000	2	2	1.000
Gastrointestinal	1	0	1.000	1	1	1.000	1	1	1.000	1	1	1.000
General aches	1	0	1.000	0	0	-	1	0	1.000	1	0	1.000

4.5.4 Evaluation of total protection conferred by Vi polysaccharide vaccine against typhoid fever

Typhoid fever occurred throughout the follow-up period, with no consistent seasonality, though increases in the caseload occurred in the autumn of 2004. This can be seen from the Kaplan-Meier curve and from the graph showing the occurrence of first episodes of typhoid fever among recipients of either vaccine during the two years of follow-up (Figure 4-4, Figure 4-5).

A total of 112 *S*. Typhi episodes were detected during the two years of follow up. 79 *S*. Typhi episodes had received a vaccine according to the study vaccination records and were used for the analysis of total protection (Table 4-4). Typhoid fever was diagnosed in 30 Vi polysaccharide vaccine recipients and 49 Hepatitis A vaccine recipients with an unadjusted total Vi polysaccharide vaccine protection of 35% (95%CI: - 20%, 65%, P=0.17) and an adjusted total protective efficacy of 29% (95%CI: -28%, 63%, P=0.24).

The sub-group analyses by age-group, gender, area of residence and vaccination coverage of the clusters were also conducted. There was no significant difference, except age-groups in the vaccine total protection by different levels of the variables mentioned. The unadjusted total vaccine protective efficacy was -30% (95%CI: -183%, 40%, P=0.51) for children age 2 to 5 years and -38% (95%CI: -192%, 35%, P=0.40) in the adjusted model (Table 4-5). Whereas for children 5 years and older the vaccine showed significant protection of 59% (95%CI: 9%, 81%, P<0.05) in the unadjusted model and 58% (95%CI: 8%, 81%, P<0.05) in the adjusted model (Table 4-6).

Figure 4-4: Occurrence of first episodes of culture confirmed typhoid fever by month during the follow up period for the Karachi Vi effectiveness trial

Vaccination took place in Sultanabad and Hijrat Colonies in August 2003 and in Bilal Colony in July 2004. Follow up was done for 2 years.

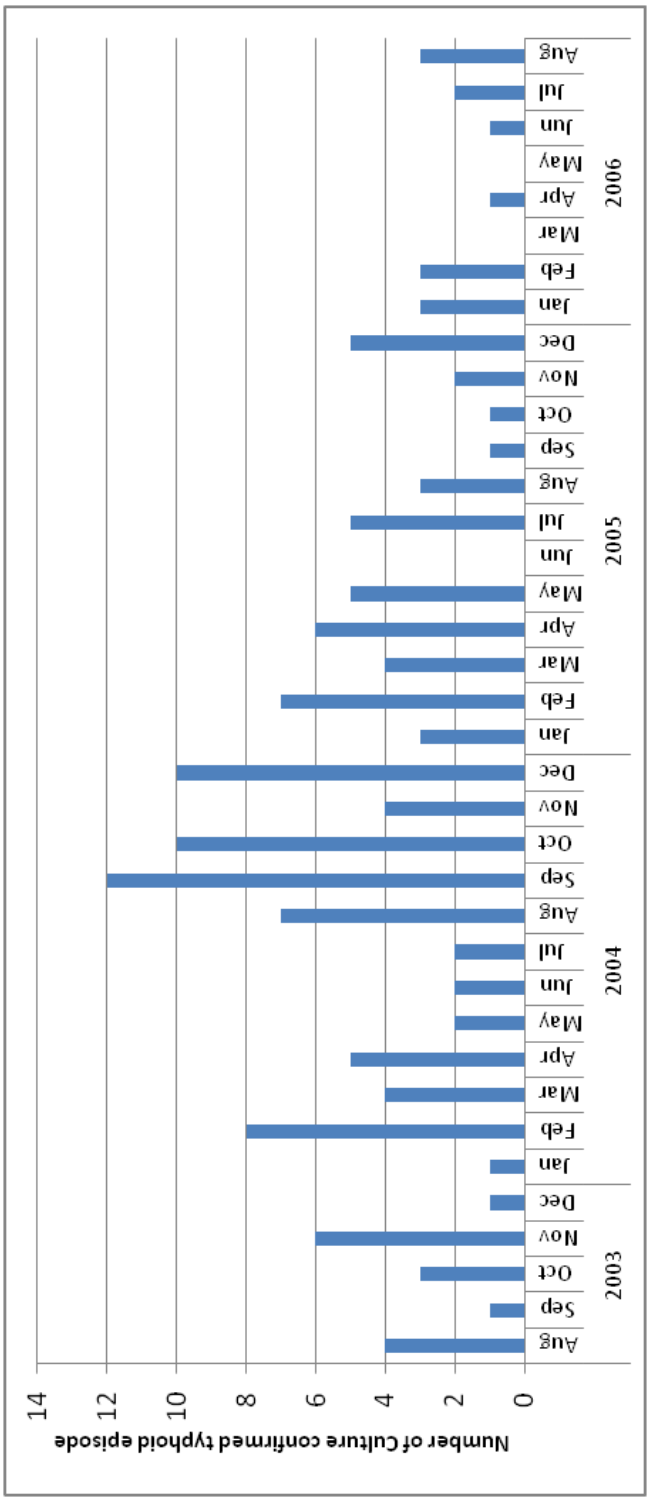


Figure 4-5: Kaplan Meier Curve of the Karachi Vi effectiveness trial followed up for two years

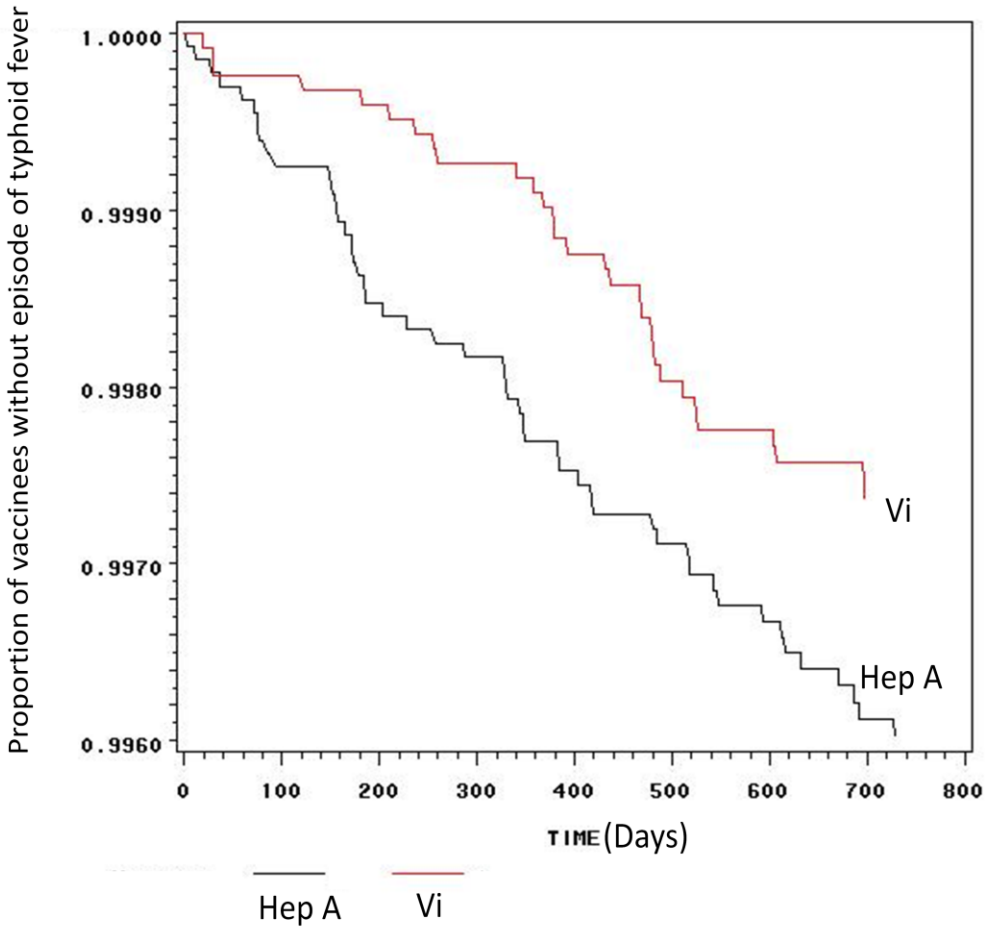


Table 4-4: Vaccine protection against typhoid in Karachi, Pakistan, amongst all vaccinees (Total Protection)

Randomized population (n=27,231)

Events	Frequency		Rate*	
	Vi (n=13,238)	Hep A (n=13,993)	Vi	Hep A
Typhoid cases[†]	30	49	0.3579	0.5515
Migration-out of study area	3354	3620	40.0140	40.7433
Deaths	11	13	0.1312	0.1463
Person-days[§]	8382068	8884898		
Average of Person-days	633.1824	634.9530		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.6491	0.3511	1.2002	0.1682

Note: n. of observations used =27,231 / n. of cases used =79

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.7038	0.3875	1.2782	0.2485
Individuals living in larger cluster specified in the stratification[11)]	0.6823	0.3858	1.2068	0.1889
Residence: Sultanabad vs. Bilal Colony	4.5661	2.4616	8.4698	0.0000
Residence: Hijrat vs. Bilal Colony	1.8645	0.7619	4.5625	0.1724
Per-capita monthly income[6)] of the household (PKR)	0.9999	0.9998	1.0000	0.0512
Age (years)	0.8806	0.8178	0.9482	0.0008

Note: n. of observations used =25,360 / n. of cases used =74

[†]Refer to footnotes of table in the previous section for description of the variables

Table 4-5: Vaccine protection against typhoid in Karachi, Pakistan, amongst all vaccinees aged 2 to 4 years (Total Protection)

Randomized population (n=6,478)

Events	Frequency		Rate*	
	Vi (n=3,154)	Hep A (n=3,324)	Vi	Hep A
Typhoid cases[†]	16	13	0.8115	0.6245
Migration-out of study area	896	943	45.4441	45.3000
Deaths	3	3	0.1522	0.1441
Person-days[§]	1971654	2081676		
Average of Person-days	625.1281	626.2563		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	1.2988	0.5952	2.8341	0.5114

Note: n. of observations used =6,478 / n. of cases used =29

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables†	HR	95% CI		p-value
		Lower	Upper	
Vaccine	1.3816	0.6531	2.9228	0.3978
Individuals living in larger cluster specified in the stratification[11)]	0.8244	0.3975	1.7095	0.6037
Residence: Sultanabad vs. Bilal Colony	2.5523	1.1017	5.9133	0.0288
Residence: Hijrat vs. Bilal Colony	0.8553	0.3280	2.2304	0.7492
Gender (0:female; 1:male)	0.5035	0.2653	0.9555	0.0358
Individuals living in a household with household head having any education[1)]	2.2983	1.1473	4.6039	0.0189
No. of total individuals in the household	1.0542	1.0048	1.1061	0.0310

Note: n. of observations used =6,478 / n. of cases used =29

[†]Refer to footnotes of table in the previous section for description of the variables

Table 4-6: Vaccine protection against typhoid in Karachi, Pakistan, amongst all vaccinees aged 5 years and above (Total Protection)

Randomized population (n=20,753)

Events	Frequency		Rate*	
	Vi (n=10,084)	Hep A (n=10,669)	Vi	Hep A
Typhoid cases[†]	14	36	0.2184	0.5292
Migration-out of study area	2458	2677	38.3439	39.3490
Deaths	8	10	0.1248	0.1470
Person-days[§]	6410414	6803222		
Average of Person-days	635.7015	637.6626		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4135	0.1872	0.9133	0.0290

Note: n. of observations used =20,753 / n. of cases used =50

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4193	0.1905	0.9232	0.0309
Individuals living in larger cluster specified in the stratification[11)]	0.6072	0.2862	1.2884	0.1937
Residence: Sultanabad vs. Bilal Colony	3.4995	1.4966	8.1832	0.0038
Residence: Hijrat vs. Bilal Colony	1.5958	0.5291	4.8126	0.4066
Monthly household income (PKR)	0.9998	0.9997	1.0000	0.0518
Higher cluster population density[8)] /100m^2	2.1086	0.9434	4.7126	0.0691

Note: n. of observations used =19,311 / n. of cases used =47

[†]Refer to footnotes of table in the previous section for description of the variables

4.5.5 Assessment of indirect and overall protection by Vi polysaccharide vaccine against typhoid fever

The level of vaccine coverage of the clusters was 52% (52% in the Vi polysaccharide vaccine clusters and 53% in the hepatitis A vaccine clusters). To evaluate indirect protection of this level of coverage by Vi polysaccharide vaccine against typhoid fever, the incidence of typhoid fever among non-recipients of Vi in clusters assigned to Vi was compared with the incidence of typhoid among non-recipients of hepatitis A vaccine in clusters assigned to this vaccine. The baseline characteristics of these non-recipients were similar (Table 4-7, Table 4-8). As shown in Table 4-9, simple (PE= 2%; 95%CI: -98%, 52%; P=0.95) and adjusted (PE= -0.8%; 95%CI: -102%, 50%; P=0.98) analyses no indirect protection was conferred by Vi polysaccharide vaccine. Overall protection by Vi polysaccharide vaccine was evaluated by comparing typhoid incidence among all members of clusters assigned to Vi polysaccharide vaccine versus all members of clusters assigned to hepatitis A vaccine. The baseline characteristics of these cohorts were similar (Table 4-10, Table 4-11). The overall protection was 27% (95%CI: -24%, 57%; P=0.24) in the simple analysis and 25% (95%CI: -20%, 53%; P=0.23) in the adjusted analysis (Table 4-12).

Table 4-7: Characteristics of the non-vaccinee population at *zero time*, Karachi, Pakistan

Variables	Vaccine cluster group		p-value*
	Vi (n=12,231)	Hep A (n=12,498)	
Individual Level			
Mean (SD)† of age (years)	8.85 (4.19)	8.85 (4.23)	0.97
No. (%) of children aged less than 5 years	2,994 (24.48)	3,157 (25.26)	0.19
No. (%) of male	6,410 (52.41)	6,690 (53.53)	0.12
Household Level			
No. (%) of individuals living in a household with household head having any education[1)]	4,901 (40.07)	5,231 (41.85)	0.32
No. (%) of individuals living in a household using safe toilet[2)]	10,073 (82.38)	10,815 (86.60)	0.14
No. (%) of individuals living in a household using safe water source[3)]	4,951 (40.57)	4,903 (39.26)	0.65
No. (%) of individuals living in owned house	6,073 (49.67)	6,076 (48.62)	0.85
No. (%) of individuals living in a household owning refrigerator	2,459 (20.10)	2,906 (23.25)	0.36
No. (%) of individuals living in a household owning motorbike	663 (5.42)	589 (4.71)	0.64
No. (%) of individuals living in a household owning washing machine	4,686 (38.31)	5,382 (43.06)	0.15
No. (%) of individuals living in a household owning at least one luxury items[4)]	5,234 (42.79)	6,026 (48.22)	0.12
Mean (SD)† monthly household income (PKR)	5761.94 (4232.0)	6083.92 (5144.5)	0.12
No. (%) of individuals with high monthly household income[5)]	4,293 (37.26)	4,558 (39.80)	0.44
Mean (SD)† per-capita monthly income[6)] of the household (PKR)	859.35 (789.38)	928.76 (1099.3)	0.03
No. (%) of individuals with high per-capita monthly income[5)] of the household	5,128 (44.50)	5,593 (48.83)	0.15
Mean (SD)† total individuals in the household	8.34 (4.58)	8.56 (5.11)	0.32
Mean (SD)† distance (m) from household to the project health centre	403.66 (221.35)	372.57 (199.45)	0.40
No. (%) of individuals living in household longer distance from the household to the project health centre[7)]	6,929 (56.65)	5,432 (43.46)	0.28
Cluster Level			
Mean (SD) number of households in the cluster	162.76 (38.26)	169.06 (38.52)	0.46
Mean (SD) number of total individuals in the cluster	1071.62 (257.28)	1121.27 (277.00)	0.33
Mean (SD) number of age eligible individuals in the cluster	461.98 (114.76)	475.78 (117.36)	0.42
Mean (SD) number of vaccinees in the cluster	229.43 (67.31)	242.07 (69.50)	0.30
Mean (SD) cluster population density /100m^2 (total individuals in the cluster/cluster area)	8.46 (3.48)	8.86 (4.13)	0.56
No. (%) of individuals living in higher cluster population density[8)]/100m^2	6,475 (52.94)	5,740 (45.93)	0.36
Mean (SD) cluster IR[9)] during 12 months before vaccination	2.38 (2.74)	3.18 (3.17)	0.24
No. (%) of individuals living in higher cluster IR during 12 months before vaccination[10)]	5,496 (44.94)	6,620 (52.97)	0.58
No. (%) of individuals living in large cluster specified in the stratification[11)]	7,285 (59.56)	7,635 (61.09)	0.72

Variables	Vaccine cluster group		p-value*
	Vi (n=12,231)	Hep A (n=12,498)	
Colony Level			
No. (%) of individuals living in Sultanabad Colony	2,204 (18.02)	2,300 (18.40)	1.00
No. (%) of individuals living in Hijrat Colony	2,685 (21.95)	2,959 (23.68)	1.00
No. (%) of individuals living in Bilal Colony	7,342 (60.03)	7,239 (57.92)	1.00

* The p-values are derived by comparing the differences between the two groups adjusted for cluster effects (Using GEE with the logit link function for dichotomous variables and the identity link function for dimensional variables).

† Mean and standard deviation (SD) was adjusted for cluster effects.

1) Any education: any type of education is counted; compared with "no education"

2) Safe toilet: use of own (non-shared) flush toilet

3) Safe water source: consumption of tap (communal/personal) water or bottled water

4) Luxury items: Refrigerator, motorbike, and/or washing machine

5) High (household/per-capita household) income: More than the median household/per-capita household income; median derived using the unvaccinated population [0: low income; 1: high income]

6) Per-capita household income: calculated from total household income divided by the number of active members in the household prior to vaccination (1USD = 60 Pakistan Rupees)

7) Longer distance to the project health centre: more than the median distance to the project health centre; median derived using the unvaccinated population [0: close to the project health centre; 1: long distance to the project health centre]

8) Higher population density: more than the median cluster population density; median derived using the unvaccinated population [0: low cluster population density; 1: high cluster population density]

9) Cluster IR: calculated from the number of cases between 366 days to 1 day prior to the 1st day of vaccination in each site (1: Sultanabad and Hijrat, 2: Bilal Colony) divided by the number of total population at ZT

10) Higher cluster incidence rate during 12 months before vaccination: more than the median incidence rate during 12 months before vaccination [0: low cluster IR; 1: high cluster IR]

11) Larger/Small cluster specified in stratification:

Sultanabad/Hijrat:	Large: >350	Small: ≤350
Bilal	Large: >450	Small: ≤450

Table 4-8: Characteristics of the clusters at *zero time* for non-vaccinee population, Karachi, Pakistan.

Randomized population (n=24,729) unless otherwise noted

Variables[†]	Vi cluster (clusters=60)	Hep A cluster (clusters=60)	Total (clusters=120)	p-value*
Mean (SD; min., max.) number of households in the cluster	153.82 (37.79; 91.00, 301.00)	158.97 (39.07; 74.00, 253.00)	156.39 (38.36; 74.00, 301.00)	0.46
Median number of households in the cluster	151.00	156.50	153.50	0.40
Mean (SD; min., max.) number of total individuals in the cluster	1000.85 (259.17; 531.00, 1936.00)	1047.38 (273.55; 519.00, 1836.00)	1024.12 (266.36; 519.00, 1936.00)	0.34
Median number of total individuals in the cluster	989.00	1016.50	998.00	0.34
Mean (SD; min., max.) number of age eligible individuals in the cluster	424.52 (118.55; 175.00, 728.00)	441.57 (117.27; 199.00, 735.00)	433.04 (117.73; 175.00, 735.00)	0.43
Median number of age eligible individuals in the cluster	410.50	434.50	419.50	0.50
Mean (SD; min., max.) number of vaccinees in the cluster	220.63 (66.87; 92.00, 458.00)	233.22 (68.13; 117.00, 459.00)	226.93 (67.51; 92.00, 459.00)	0.31
Median number of vaccinees in the cluster	214.50	222.50	219.50	0.28
Mean (SD; min., max.) cluster population density/100m^2	8.98 (3.62; 2.85, 19.06)	9.41 (4.44; 3.52, 19.63)	9.19 (4.04; 2.85, 19.63)	0.56
Median cluster population density/100m^2	8.34	7.81	8.13	0.91
Mean (SD; min., max.) cluster IR[9)] during 12 months before vaccination	2.27 (2.73; 0.00, 8.99)	2.90 (3.16; 0.00, 11.09)	2.59 (2.95; 0.00, 11.09)	0.24
Median cluster IR[9)] during 12 months before vaccination	1.64	2.25	2.01	0.29
Mean (SD; min., max.) cluster vaccine coverage[12)]	52.64 (9.55; 30.70, 68.44)	53.35 (9.44; 35.53, 79.21)	52.99 (9.46; 30.70, 79.21)	0.68
Median cluster vaccine coverage[12)]	52.82	52.41	52.48	0.98
Number of small clusters[11)]	31	29	60	0.72
Number of large clusters[11)]	29	31	60	.

*The p-values are derived by comparing the differences between the two groups (t-test for mean comparison, chi-square test for categorical variable, and non-parametric method (Wilcoxon rank sum test) for median comparison).

[†]Refer to footnotes of table in the previous section for description of the variables

[12)] Cluster vaccine coverage was derived from population aged 2.0 to 16.0 years

Table 4-9: Vaccine protection against typhoid for non-vaccinee population, Karachi, Pakistan.

Randomized population (n=24,729)

Events	Frequency		Rate*	
	Vi (n=12,231)	Hep A (n=12,498)	Vi	Hep A
Typhoid cases[†]	15	16	0.2294	0.2354
Migration-out of study area	5412	5533	82.7507	81.3986
Deaths	13	19	0.1988	0.2795
Person-days[§]	6540126	6797411		
Average of Person-days	534.7172	543.8799		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.9758	0.4794	1.9859	0.9461

Note: n. of observations used =24,729 / n. of cases used =31

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	1.0084	0.5046	2.0151	0.9812
Individuals living in larger cluster specified in the stratification[11)]	0.2605	0.0889	0.7632	0.0142
Residence: Sultanabad vs. Bilal Colony	2.7785	0.9976	7.7386	0.0505
Residence: Hijrat vs. Bilal Colony	1.3962	0.6290	3.0994	0.4120
Individuals living in owned house	0.5143	0.2345	1.1280	0.0971
Individuals living in a household owning motorbike	2.9708	0.9731	9.0693	0.0559
Individuals with high per-capita monthly income[5)] of the household	2.2012	1.0350	4.6814	0.0404
No. of vaccinees in the cluster	1.0095	1.0039	1.0151	0.0009

Note: n. of observations used =22,958 / n. of cases used =29

[†]Refer to footnotes of table in the previous section for description of the variables

Table 4-10: Characteristics of the study population at zero time, Karachi, Pakistan

Variables	Vaccine group		p-value*
	Vi (n=25,471)	Hep A (n=26,494)	
Individual Level			
Mean (SD)† of age (years)	8.59 (4.00)	8.62 (4.01)	0.58
No. (%) of children aged less than 5 years	6,150 (24.15)	6,482 (24.47)	0.45
No. (%) of male	13,311 (52.26)	13,883 (52.40)	0.76
Household Level			
No. (%) of individuals living in a household with household head having any education[1)]	10,306 (40.46)	11,274 (42.55)	0.23
No. (%) of individuals living in a household using safe toilet[2)]	21,008 (82.54)	22,770 (86.02)	0.22
No. (%) of individuals living in a household using safe water source[3)]	10,291 (40.47)	10,100 (38.14)	0.64
No. (%) of individuals living in owned house	14,971 (58.80)	15,179 (57.33)	0.40
No. (%) of individuals living in a household owning refrigerator	5,904 (23.18)	6,759 (25.51)	0.46
No. (%) of individuals living in a household owning motorbike	1,473 (5.78)	1,434 (5.41)	0.80
No. (%) of individuals living in a household owning washing machine	10,624 (41.71)	12,161 (45.90)	0.22
No. (%) of individuals living in a household owning at least one luxury items[4)]	11,815 (46.39)	13,458 (50.80)	0.20
Mean (SD) † monthly household income (PKR)	5714.30 (4475.7)	5938.74 (4780.6)	0.24
No. (%) of individuals with high monthly household income[5)]	8,942 (37.11)	9,465 (39.02)	0.43
Mean (SD) † per-capita monthly income[6)] of the household (PKR)	778.95 (688.11)	816.32 (896.39)	0.09
No. (%) of individuals with high per-capita monthly income[5)] of the household	11,318 (46.96)	12,134 (50.02)	0.15
Mean (SD) † total individuals in the household	8.73 (4.63)	8.87 (4.93)	0.46
Mean (SD) † distance (m) from household to the project health centre	402.60 (220.90)	373.03 (5673.7)	0.43
No. (%) of individuals living in household longer distance from the household to the project health center[7)]	14,105 (55.38)	11,877 (44.83)	0.28
Cluster Level			
Mean (SD) number of households in the cluster	162.75 (39.91)	167.88 (39.25)	0.46
Mean (SD) number of total individuals in the cluster	1066.73 (267.77)	1114.17 (281.54)	0.33
Mean (SD) number of age eligible individuals in the cluster	457.07 (117.65)	472.19 (120.09)	0.42
Mean (SD) number of vaccinees in the cluster	235.21 (69.70)	247.72 (72.25)	0.30
Mean (SD) cluster population density /100m^2 (total individuals in the cluster/cluster area)	8.68 (3.53)	9.20 (4.29)	0.56
No. (%) of individuals living in higher cluster population density[8)]/100m^2	14,107 (55.38)	11,660 (44.01)	0.20
Mean (SD) cluster IR[9)] during 12 months before vaccination	2.36 (2.72)	3.02 (3.12)	0.24
No. (%) of individuals living in higher cluster IR during 12 months before vaccination[10)]	11,529 (45.26)	14,165 (53.46)	0.47
No. (%) of individuals living in large cluster specified in the stratification[11)]	14,867 (58.37)	16,059 (60.61)	0.72
Colony Level			

Variables	Vaccine group		p-value*
	Vi (n=25,471)	Hep A (n=26,494)	
No. (%) of individuals living in Sultanabad Colony	4,876 (19.14)	5,297 (19.99)	1.00
No. (%) of individuals living in Hijrat Colony	6,318 (24.80)	6,486 (24.48)	1.00
No. (%) of individuals living in Bilal Colony	14,277 (56.05)	14,711 (55.53)	1.00

* The p-values are derived by comparing the differences between the two groups adjusted for cluster effects (Using GEE with the logit link function for dichotomous variables and the identity link function for dimensional variables).

† Mean and standard deviation (SD) was adjusted for cluster effects.

1) Any education: any type of education is counted; compared with “no education”

2) Safe toilet: use of own (non-shared) flush toilet

3) Safe water source: consumption of tap (communal/personal) water or bottled water

4) Luxury items: Refrigerator, motorbike, and/or washing machine

5) High (household/per-capita household) income: More than the median household/per-capita household income; median derived using the entire population [0: low income; 1: high income]

6) Per-capita household income: calculated from total household income divided by the number of active members in the household prior to vaccination (1USD = 60 Pakistan Rupees)

7) Longer distance to the project health centre: more than the median distance to the project health centre; median derived using the entire population [0: close to the project health centre; 1: long distance to the project health centre]

8) Higher population density: more than the median cluster population density; median derived using the entire population [0: low cluster population density; 1: high cluster population density]

9) Cluster IR: calculated from the number of cases between 366 days to 1 day prior to the 1st day of vaccination in each site (1: Sultanabad and Hijrat, 2: Bilal Colony) divided by the number of total population at ZT

10) Higher cluster incidence rate during 12 months before vaccination: more than the median incidence rate during 12 months before vaccination [0: low cluster IR; 1: high cluster IR]

11) Larger/Small cluster specified in stratification:

Sultanabad/Hijrat:	Large: >350	Small: ≤350
Bilal	Large: >450	Small: ≤450

Table 4-11: Characteristics of the clusters at *zero time* for all study population, Karachi, Pakistan.

Randomized population (n=51,965) unless otherwise noted

Variables[†]	Vi (clusters=60)	Hep A (clusters=60)	Total (clusters=120)	p-value*
Mean (SD; min., max.) number of households in the cluster	153.82 (37.79; 91.00, 301.00)	158.97 (39.07; 74.00, 253.00)	156.39 (38.36; 74.00, 301.00)	0.46
Median number of households in the cluster	151.00	156.50	153.50	0.40
Mean (SD; min., max.) number of total individuals in the cluster	1000.85 (259.17; 531.00, 1936.00)	1047.38 (273.55; 519.00, 1836.00)	1024.12 (266.36; 519.00, 1936.00)	0.34
Median number of total individuals in the cluster	989.00	1016.50	998.00	0.34
Mean (SD; min., max.) number of age eligible individuals in the cluster	424.52 (118.55; 175.00, 728.00)	441.57 (117.27; 199.00, 735.00)	433.04 (117.73; 175.00, 735.00)	0.43
Median number of age eligible individuals in the cluster	410.50	434.50	419.50	0.50
Mean (SD; min., max.) number of vaccinees in the cluster	220.63 (66.87; 92.00, 458.00)	233.22 (68.13; 117.00, 459.00)	226.93 (67.51; 92.00, 459.00)	0.31
Median number of vaccinees in the cluster	214.50	222.50	219.50	0.28
Mean (SD; min., max.) cluster population density/100m^2	8.98 (3.62; 2.85, 19.06)	9.41 (4.44; 3.52, 19.63)	9.19 (4.04; 2.85, 19.63)	0.56
Median cluster population density/100m^2	8.34	7.81	8.13	0.91
Mean (SD; min., max.) cluster IR[9)] during 12 months before vaccination	2.27 (2.73; 0.00, 8.99)	2.90 (3.16; 0.00, 11.09)	2.59 (2.95; 0.00, 11.09)	0.24
Median cluster IR[9)] during 12 months before vaccination	1.64	2.25	2.01	0.29
Mean (SD; min., max.) cluster vaccine coverage[12)]	52.64 (9.55; 30.70, 68.44)	53.35 (9.44; 35.53, 79.21)	52.99 (9.46; 30.70, 79.21)	0.68
Median cluster vaccine coverage[12)]	52.82	52.41	52.48	0.98
Number of small clusters[11)]	31	29	60	0.72
Number of large clusters[11)]	29	31	60	.

*The p-values are derived by comparing the differences between the two groups (t-test for mean comparison, chi-square test for categorical variable, and non-parametric method (Wilcoxon rank sum test) for median comparison).

[†]Refer to footnotes of table in the previous section for description of the variables

[12)] Cluster vaccine coverage was derived from population aged 2.0 to 16.0 years

Table 4-12: Vaccine protection against typhoid for all study population, Karachi, Pakistan.

Randomized population (n=51,965)

Events	Frequency		Rate*	
	Vi (n=25,471)	Hep A (n=26,494)	Vi	Hep A
Typhoid cases[†]	45	65	0.3016	0.4145
Migration-out of study area	8768	9156	58.7581	58.3843
Deaths	24	32	0.1608	0.2041
Person-days[§]	14922194	15682309		
Average of Person-days	585.8503	591.9193		

*Rate = no. of events / person-days X 100,000

[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.7278	0.4271	1.2400	0.2425

Note: n. of observations used =51,965 / n. of cases used =110

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.7490	0.4667	1.2022	0.2313
Individuals living in larger cluster specified in the stratification[11)]	0.7393	0.4676	1.1691	0.1965
Residence: Sultanabad vs. Bilal Colony	3.4229	2.0942	5.5946	0.0000
Residence: Hijrat vs. Bilal Colony	1.5545	0.7873	3.0694	0.2037
Individuals living in a household using safe water source[3)]	0.7129	0.5016	1.0131	0.0591
Individuals living in a household using safe toilet[2)]	2.2037	1.1762	4.1289	0.0136
Age group (0: <5.0y; 1: ≥5.0y)	0.5792	0.3670	0.9143	0.0190

Note: n. of observations used =51,877 / n. of cases used =110

[†]Refer to footnotes of table in the previous section for description of the variables

4.5.6 Evaluation of protection conferred by Vi polysaccharide vaccine against paratyphoid fever

To assess whether the evaluation of protection by Vi polysaccharide vaccine against typhoid fever could have been biased, we analyzed Vi protection against enteric fever due to *S.* Paratyphi A. Paratyphoid fever caused by *S.* Paratyphi A was diagnosed in 43 subjects in the Vi polysaccharide vaccine group, as compared with 34 subjects in the hepatitis A vaccine group, resulting in a negative protective effectiveness for the Vi polysaccharide vaccine of -34% (95%CI: -137%, 24%, P=0.31) in the simple analysis and of -37% (95%CI: -138%, 21%; P = 0.26) in the adjusted analysis (Table 4-13).

Table 4-13: Vaccine protection against paratyphoid in Karachi, Pakistan

Randomized population (n=27,231)

Events	Frequency		Rate*	
	Vi (n=13,238)	Hep A (n=13,993)	Vi	Hep A
Paratyphoid cases[†]	43	34	0.5135	0.3825
Migration-out of study area	3359	3628	40.1092	40.8099
Deaths	11	14	0.1313	0.1575
Person-days[§]	8374642	8889999		
Average of Person-days	632.6214	635.3176		

*Rate = no. of events / person-days X 100,000

[†]A paratyphoid case is defined by culture positive for *S.* Paratyphi A or B and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period

[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	1.3435	0.7601	2.3745	0.3096

Note: n. of observations used =27,231 / n. of cases used =77

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	1.3704	0.7881	2.3829	0.2643
Individuals living in larger cluster specified in the stratification[11)]	1.1421	0.7003	1.8626	0.5945
Residence: Sultanabad vs. Bilal Colony	0.8190	0.3953	1.6969	0.5911
Residence: Hijrat vs. Bilal Colony	2.3904	1.3685	4.1756	0.0022
Gender (0:female; 1:male)	1.6899	1.0901	2.6199	0.0190
Individuals with high monthly household income[5)]	0.5918	0.3463	1.0113	0.0550

Note: n. of observations used =25,376 / n. of cases used =73

[†]Refer to footnotes of table in the previous section for description of the variables

4.6 Discussion

The use of Vi polysaccharide vaccine in Karachi, Pakistan resulted in significant protection (PE=57%; 95%CI: 6%, 81%) for children 5 to 16 years of typhoid fever and was associated with minimal vaccine related adverse events. We did not find any statistically significant indirect or overall protection for the same population.

The data on Vi polysaccharide vaccine efficacy for children 5 to 16 years are consistent with earlier trials of Vi polysaccharide vaccine (Acharya, Lowe et al. 1987; Klugman, Gilbertson et al. 1987; Klugman, Koornhof et al. 1996; Sur, Ochiai et al. 2009). We believe the study provides unbiased estimates of Vi polysaccharide vaccine efficacy for this age group. We also assessed effectiveness of Vi polysaccharide vaccine in preventing paratyphoid fever as Vi polysaccharide vaccine does not protect against *S*. Paratyphi A. Paratyphoid fever caused by *S.* Paratyphi A was diagnosed in 43 subjects in the Vi polysaccharide vaccine group, as compared with 34 subjects in the hepatitis A vaccine group, suggesting that the estimates provide sufficient evidence that the results have minimal bias in the estimates of Vi polysaccharide vaccine efficacy. Although not statistically significant, larger number of cases identified from the Vi polysaccharide vaccine arm concerns the hypothesis of potential increase of paratyphoid cases upon introduction of typhoid vaccine similar to what is known as serotype replacement for some other diseases. Such phenomenon must be observed for all areas where typhoid vaccine is introduced.

In the surveillance for enteric fever we identified 77 cases of blood culture diagnosed *S*. Paratyphi A. Although there was no significant difference in the incidence of paratyphoid between the Vi clusters and the Hepatitis A clusters, the results highlight the importance of *S.* Paratyphi infection in children. Increase in the *S*. Paratyphi incidence as a result of typhoid fever vaccination has not been seen, but Vi vaccination does not cross protect against Paratyphi A and B, the two major serotypes found in the typhoid endemic settings. The impact of Vi vaccination on overall burden of enteric fever is a limitation on its use. A bivalent vaccine having Vi and S. Paratyphi A may well reduce the burden of enteric fever in children.

4.6.1 Potential limitations

Similar to all epidemiological studies we have following limitation in the trial to report as follows: i) the dropout rate in the study population was approximately 25%, higher than the expected 10% that was used for sample size calculation, affecting the power of the study to detect a statistically significant result; ii) the study population was highly mobile with frequent movement within the study settings increasing the chances of contamination and thus affecting the ability to estimate the indirect and overall effect of the Vi polysaccharide vaccine; iii) all efforts were made to ensure proper identification at the time of vaccination, at the time on enrolment in surveillance, confirming the residence after a *S.* Typhi case was identified in the study health centre and at the time of household visit to collect member and household information. The data collection teams at each level were qualified and highly trained. However, errors may have occurred whereby due to incorrect identification we might have misclassified study participants; and iv) the study established health centre to provide health services to febrile illnesses in the catchment area. The intention was also not to change the health seeking behaviour of the study population. Approximately 150 private health clinics were operated by other health care providers. We established a referral system for febrile episodes, however not all providers participated in the surveillance. Therefore we might have missed cases of typhoid fever who visited providers outside study setting or from those who did not participate in the referral system.

The study population belonged to the low socio-economic quartile compared to the rest of the Karachi. A major proportion of the study population continuously moved within and outside the study setting. Among the vaccinated population 6,084 (22%) vaccinated children moved out of the study setting. in addition another 910 (3%) moved out of their cluster of randomization. The migration rates were not statistically significantly different between the two arms of the study. We had inflated the target sample size with an additional 10% for loss to follow up; however, we lost more subjects due to migration. The 25% loss to follow up would have potentially affected the power of detecting a significant difference of Vi polysaccharide vaccine efficacy. The study site was densely populated with a population density of 12 people per $100m^2$. The random mixing of population could also have resulted in the contamination of vaccinated clusters with high presence of non-vaccinated children and

thus increases exposure to the *S*. Typhi, and a net effect on moderate efficacy of Vi polysaccharide vaccine.

4.6.2 Implications

The burden of typhoid fever in children of urban squatter settlements requires a comprehensive approach for the control of the disease. It was assessed and found that the risk of typhoid fever in low socio-economic settings is a result of household practice of safe drinking water and increased congestion due to higher number of household members in Pakistan (Luby, Faizan et al. 1998). The 57% protective efficacy of Vi polysaccharide vaccine in settings like the study site, along with water purification measure can have a synergistic effect on the control of the disease. Typhoid control has been possible with such approaches in countries like Thailand with the whole cell killed vaccine in the 1970's (Bodhidatta, Taylor et al. 1987). In addition, the evidence from Kolkata on indirect protection conferred by Vi polysaccharide vaccine can be adopted for population beyond the paediatric age in the high risk geographic settings of urban Pakistan. A school-based Vi vaccination programme is shown to be feasible (Agtini, Ochiai et al. 2006; Thiem, Danovaro-Holliday et al. 2006). A similar programme in Pakistan may have a significant impact on burden of typhoid fever in children in a short period of time.

5 COMPARISON OF CLINICAL PROTECTION BY AGE GROUP FROM KOLKATA AND KARACHI EFFECTIVENESS TRIALS

5.1 Introduction

The DOMI Programme conducted Vi polysaccharide vaccine effectiveness trails in Karachi, Pakistan, targeting children 2 to 16 years old, and in Kolkata, India, targeting all those who are 2 years old or older to assess the feasibility of introduction Vi polysaccharide vaccine as a regular public health programme as described in earlier chapters. The protective effectiveness (PE) using clinical protection as endpoint showed that the vaccine protected those who are aged 5-15 years equally from both sites (Kolkata: PE=56% [95%CI: 18%, 77%]; Karachi: PE=58% [95%CI: 8%, 81%]), however, the PE were very different among the pre-school children aged 2-4 years of age (Kolkata: PE=80% [95%CI: 53%, 91%]; Karachi: PE=-38% [95%CI: -192%, 35%]).

The difference in the effectiveness estimates in younger children between the studies in the sites in Karachi and Kolkata could be due to the difference in study design and the population characteristics. The study in Kolkata included a population older than 2 years including adults, whereas in Karachi, only children were targeted for the vaccination. Therefore the net vaccinated population in Kolkata was much higher than in Karachi (coverage of 60% in Kolkata vs. 22% of total population of the sites). Indirect protection in younger children could have been potentiated by the effect of reduced transmission and hence lower circulation of *S*. Typhi in the environment (Halloran, Haber et al. 1991). The resultant reduced exposure of younger children to *S*. Typhi could have played a major role in reducing the incidence of typhoid fever in the vaccinated clusters of Kolkata. Furthermore, it is worthwhile to note that the dropout rate from the Karachi effectiveness trial was significantly more than what had initially be expected for the sample size calculation, resulting in substantial loss of power to detect the protective effectiveness from the study population. This was not the case in Kolkata site.

In order to review and hypothesize the differences in the protection in young children, epidemiological data from 2 sites were contrasted. If the protection in the young children in Kolkata site had been due to the fact much larger population has been vaccinated in the community and induced indirect protection to this age group, unvaccinated children in Kolkata site should be protected indirectly (or to show indication of indirect protection). On the other hand, if such indirect protection was not present in Karachi site due to the lower community-level coverage, younger children in Karachi site should observe no or random protective level similar to the vaccinated children of the same age group. In this chapter the comparison of the protective results by age group are described. Note that this is a *post hoc* analysis and not stipulated in the initial analytical plan.

5.2 Methods

The same dataset from the Kolkata and Karachi effectiveness trials have been used in *post hoc* fashion to describe the protection by age groups.

Vaccine protection by age groups was assessed in several ways. In each approach, enteric fever episode outcomes were analyzed if their onset occurred from the day after zero time, and persons were analyzed according to the vaccine assigned to their clusters. The *total vaccine protection*, comparing rates of episodes of typhoid fever in recipients of Vi polysaccharide vaccine versus recipients of hepatitis A vaccine, has already been analyzed by age groups. We evaluated the *indirect vaccine protection* conferred by Vi polysaccharide vaccine against typhoid fever by comparing the rates of typhoid fever in non-vaccinees in clusters assigned to Vi polysaccharide vaccine versus those for non-vaccinees in the clusters assigned to hepatitis A vaccine by age group in this chapter.

To estimate vaccine protection, we used Cox proportional hazard regression models, verifying first that the proportionality assumption was satisfied for all independent variables (Lin, Wei et al. 1993). These models compared the time from zero time to the first episode of the analyzed outcome in members of the Vi polysaccharide vaccine group versus members of the hepatitis A vaccine group; censoring events for these analyses included migration out of the zero-time

cluster, death, or completion of the two-year, post-zero time follow-up, whichever came first. These were ascertained through censuses conducted during the follow up period. Relative rates (RRs) of the target outcome in the Vi versus the hepatitis A groups were estimated by exponentiating the coefficient for the vaccine variable in these models, and vaccine protective effectiveness (PE) was estimated as (1- RR) X 100%. Standard errors for the coefficients for the vaccine variable in the models were used to estimate P values and 95% confidence intervals for the RRs. Simple analyses of vaccine impact were adjusted for the design effect of the cluster randomization in these models with robust sandwich variance estimates, also known as Huber-White standard error estimates (Reid and Crèpeau 1985; Lin and Wei 1989). Final adjusted estimates were obtained from models that also included the variables used for stratified randomization, as well as potentially confounding variables independently associated with the time to the event at $P<0.1$ in a backward selection algorithm. All P values and 95% confidence intervals for these analyses were estimated in a two-tailed fashion.

5.3 Results

5.3.1 Summary of the differences in total protection by age groups

The protective effectiveness (PE) for total protection was estimated in earlier respective chapters (Table 3-6 and Table 3-6; Table 4-6 and Table 4-6). The PE using clinical protection as endpoint showed that the vaccine protected vaccinees who are aged 5-15 years equally from both sites (Kolkata: PE=56% [95%CI: 18%, 77%]; Karachi: PE=58% [95%CI: 8%, 81%]), however, the PE were different among the pre-school children aged 2-4 years of age (Kolkata: PE=80% [95%CI: 53%, 91%]; Karachi: PE=-38% [95%CI: -192%, 35%]) (Table 5-1).

Table 5-1: Summary of vaccine total protection against typhoid in Kolkata, India, and Karachi, Pakistan among vaccinated children

Total Protection	Protective Effectiveness (%)	
Age group (years)	**Kolkata (95% CI)**	**Karachi (95% CI)**
2 to 4	80% (53%, 91%)	-38% (-192%, 35%)
5 to 15 (Kolkata) 5 to 16 (Karachi)	56% (18%, 77%)	58% (8%, 81%)

5.3.2 Indirect protection by age groups

There were 7 culture confirmed cases (2 in Vi clusters and 5 in Hep A clusters) of typhoid fever episodes in age group 2 to 4 years and 18 cases (5 in Vi clusters and 13 in Hep A clusters) in age group 5 to 15 years from the Kolkata site. The estimates of indirect protection for Kolkata site by age groups were 7% (95%CI: -439%, 84%) for pre-school children aged 2 to 4 years and 55% (95%CI: -20%, 83%) for those who were aged 5 to 15 years (Table 5-2; Table 5-3).

In Karachi site, there were 10 culture confirmed cases (3 in Vi clusters and 7 in Hep A clusters) of typhoid fever episodes in age group 2 to 4 years and 21 cases (12 in Vi clusters and 9 in Hep A clusters) in age group 5 to 16 years. The estimates of indirect protection for Karachi site by age groups were 54% (95%CI: -84%, 88%) for pre-school children aged 2 to 4 years and -41% (95%CI: -259%, 43%) for those who were aged 5 to 16 years (Table 5-4; Table 5-5).

Table 5-6 summarizes the findings on indirect protection from the two sites for both crude analysis looking only the cluster effect and final models with backward elimination process considering the covariates.

Table 5-2: Vaccine indirect protection against typhoid in Kolkata, India among children aged 2 to 4 years

Randomized population (n=1,255)

Events	Frequency		Rate*	
	Vi (n=617)	Hep A (n=638)	Vi	Hep A
Typhoid cases[†]	2	5	0.4773	1.1281
Migration-out of study area	95	79	22.6734	17.8246
Deaths	1	-	0.2387	-
Person-days[§]	418993	443207		
Average of Person-days	679.0810	694.6818		

*Rate = no. of events / person-days X 100,000
[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period
[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4276	0.0794	2.3018	0.3225

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.9312	0.1607	5.3963	0.9367
Population density /100m^2 (total study population/area)	1.0504	1.0201	1.0817	0.0010
Distance (m) from the household to the nearest health outpost	0.9869	0.9777	0.9963	0.0061
Larger cluster size of adults at stratification	3.1450	0.4669	21.1844	0.2391
Larger cluster size of paediatrics at stratification	0.6086	0.0707	5.2366	0.6511
IR during 12 months before vaccination	0.4289	0.2679	0.6866	0.0004

[†]Refer to table in Chapter 3 for description of the variables.

Table 5-3: Vaccine indirect protection against typhoid in Kolkata, India among children aged 5 to 15 years

Randomized population (n=3,601)

Events	Frequency		Rate*	
	Vi (n=1,655)	Hep A (n=1,946)	Vi	Hep A
Typhoid cases[†]	5	13	0.4450	0.9780
Migration-out of study area	259	279	23.0519	20.9889
Deaths	1	2	0.0890	0.1505
Person-days[§]	1123553	1329272		
Average of Person-days	678.8840	683.0791		

*Rate = no. of events / person-days X 100,000
[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period
[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4542	0.1692	1.2192	0.1172

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4505	0.1681	1.2076	0.1130
Larger cluster size of paediatrics at stratification	0.9625	0.1909	4.8531	0.9631
Larger cluster size of adults at stratification	1.2879	0.5037	3.2929	0.5973
Individuals with high per-capita monthly expenditure	0.1443	0.0202	1.0335	0.0540
Longer distance from the household to the health outpost	0.3865	0.1389	1.0754	0.0686

[†]Refer to table in Chapter 3 for description of the variables.

Table 5-4: Vaccine indirect protection against typhoid in Karachi, Pakistan among children aged 2 to 4 years

Randomized population (n=6,151)

Events	Frequency		Rate*	
	Vi (n=2,994)	Hep A (n=3,157)	Vi	Hep A
Typhoid cases[†]	3	7	0.1911	0.4121
Migration-out of study area	1396	1462	88.9285	86.0674
Deaths	6	9	0.3822	0.5298
Person-days[§]	1569801	1698669		
Average of Person-days	524.3156	538.0643		

*Rate = no. of events / person-days X 100,000
[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period
[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4619	0.1261	1.6910	0.2434

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	0.4639	0.1164	1.8494	0.2763
Individuals living in larger cluster specified in the stratification	0.0598	0.0097	0.3677	0.0024
Residence: Sultanabad vs. Bilal Colony	2.8034	0.5144	15.2779	0.2334
Residence: Hijrat vs. Bilal Colony	0.7432	0.0712	7.7625	0.8042
Per-capita monthly income of the household (PKR)	1.0002	1.0001	1.0003	0.0022
No. of household in the cluster	1.0389	1.0175	1.0607	0.0003

[†]Refer to footnotes of table in Chapter 4 for description of the variables

Table 5-5: Vaccine indirect protection against typhoid in Karachi, Pakistan among children aged 5 to 16 years

Randomized population (n=18,578)

Events	Frequency		Rate*	
	Vi (n=9,237)	Hep A (n=9,341)	Vi	Hep A
Typhoid cases[†]	12	9	0.2414	0.1765
Migration-out of study area	4016	4071	80.7995	79.8432
Deaths	7	10	0.1408	0.1961
Person-days[§]	4970325	5098742		
Average of Person-days	538.0887	545.8454		

*Rate = no. of events / person-days X 100,000
[†]A typhoid case is defined by culture positive for *S.* Typhi and residence confirmed in subsequent follow-up visit; a person can only be infected once during the study period
[§]Based on time to the first event

Vaccine protection with adjustment of cluster effect using the PH (Proportional Hazard) model

Variable	HR	95% CI		p-value
		Lower	Upper	
Vaccine	1.3713	0.5096	3.6900	0.5318

Vaccine protection with adjustment of covariates and cluster effect using the PH model: Final model by backward elimination

Variables[†]	HR	95% CI		p-value
		Lower	Upper	
Vaccine	1.2600	0.4632	3.4272	0.6508
Individuals living in larger cluster specified in the stratification	0.6553	0.2536	1.6932	0.3828
Residence: Sultanabad vs. Bilal Colony	2.6837	0.7336	9.8172	0.1357
Residence: Hijrat vs. Bilal Colony	1.8519	0.7091	4.8366	0.2083
Individuals with high per-capita monthly income of the household	2.3396	0.9988	5.4806	0.0503
Individuals living in a household owning motorbike	3.6992	1.3252	10.3265	0.0125

[†]Refer to table in Chapter 4 for description of the variables

Table 5-6: Summary of crude vaccine indirect protection against typhoid in Kolkata, India, and Karachi, Pakistan among non-vaccinated children with adjustment of cluster effect using the Proportional Hazard model

Indirect Protection	Protective Effectiveness (%)	
Age group (years)	**Kolkata (95% CI)**	**Karachi (95% CI)**
2 to 4	57% (-130%, 92%)	54% (-69%, 87%)
5 to 15 (Kolkata) 5 to 16 (Karachi)	55% (-22%, 83%)	-37% (-269%, 49%)

Summary of vaccine indirect protection against typhoid in Kolkata, India, and Karachi, Pakistan among non-vaccinated children with adjustment of covariates and cluster effect using the Proportional Hazard model (Final model by backward elimination)

Indirect Protection	Protective Effectiveness (%)	
Age group (years)	**Kolkata (95% CI)**	**Karachi (95% CI)**
2 to 4	7% (-439%, 84%)	54% (-84%, 88%)
5 to 15 (Kolkata) 5 to 16 (Karachi)	55% (-20%, 83%)	-41% (-259%, 43%)

5.4 Discussion

Typhoid fever episodes were regularly and continuously reported to the project health facilities during the follow up period in both Kolkata and Karachi. Protection was consistent for the school-aged children, but not consistent for the younger children aged between 2 and 4 years. In case the vaccine reduced the force of infection in the community through reduced transmission of the disease in Kolkata site with higher community vaccine coverage, then we would have seen indication of, if not statistically significant, indirect protection in the younger age group of Kolkata site population and possibly absence of such protection in the same age group of Karachi site population.

The crude results not taking into account other variables show an indication of similar protection in this age group (age 2 to 4 years) from both sites. However, consideration of covariates and inclusion of backward elimination process resulted in peculiar notable reduction in indirect protective level from 2 to 4 year old children in Kolkata site. This may be due to collinearity in some of the covariates selected. This possibility of collinearity acting upon the final data was not examined in this study.

The analysis result suggests that there was no indication of indirect protection among younger children but possible indirect protection in school-going children in Kolkata site. While in Karachi site, there was an indication of indirect protection in younger children, but not in school-going children. Note that none of these results were statistically significant.

The varying results not consistent to the hypothesis suggested review of the surveillance system as a whole. We had reviewed the system by examining the case reporting and registrations. The facility staff, community, and study participants were blinded from the vaccines allocated and there were no differences in the registration, use, or referral from general practitioners to the project health facilities throughout the follow up period. Review of positive case files for this age group also did not indicate any misclassification of cases.

Looking at the incidence rate in the youngest age groups (2 to 5 years) of Karachi and Kolkata study sites, the incidence rates in the vaccine groups is almost the same

(0.81/100,000 person-days in Karachi vs. 0.64/100,000 person-days in Kolkata), however, it is very different between the control groups (0.62/100,000 person-days in Karachi vs. 3.54/100,000 person-days in Kolkata). In contrary, at different age groups, incidence rates in the older age groups of the control group is quite different in Kolkata (3.54/100,000 person-days in age 2-5 years vs. 1.67/100,000 person-days in age 5-15 years), but not so in Karachi (0.62/100,000 person-days in age 2-5 years vs. 0.53/100,000 person-days in age 5-16 years). These points also do not correlate with the pre-vaccination disease burden study. The incidence rate in the control group of the 2 to 5 year age group of Kolkata is almost 4 times higher than the pre-vaccination disease burden study (340/100,000 pre vs. 1,233/100,000 post in control group), while the incidence rate in the control group of the 5 to 15 year age group is similar to the pre-vaccination data (494/100,000 pre vs. 589/100,000 post in control group). As opposed to the increasing rate of diseases in Kolkata, Karachi site has shown a significant decrease in the incidence of typhoid. The incidence rate in the control group of the age group 2 to 5 years is less than half the pre-vaccination disease burden study (573/100,000 pre vs. 196/100,000 post in control group). The incidence rate in the control group of the age group 5 to 15 years is almost half the pre-vaccination disease burden study (413/100,000 pre vs. 169/100,000 post in control group).

The inherent limitation to the cluster randomized design is possible clustering of cases. The above incidence variation in time and place may be due to such clustering of cases, possibly rare occurrence of such breakthrough cases from a confined area or clusters. In order to assess the varying vaccine impacts in Kolkata and Karachi independently of the epidemiological differences and possible design effect, we looked into immunological differences by measuring the immunogenicity post immunization from randomly selected individuals from both vaccine and active control arms.

6 COMPARISON OF IMMUNOGENICITY TEST RESULTS FROM KOLKATA AND KARACHI EFFECTIVENESS TRIALS

6.1 Introduction

The Vi polysaccharide vaccine contains 25µg of purified Vi polysaccharide, to be administered intramuscularly. It can be administered to non-pregnant individuals two years and older (available at: http://www.msds-gsk.com/uk presc/00417009.pdf). The Vi polysaccharide is a homopolymer of 1-4, 2 deoxy-2-N-acetyl galacturonic acid and is extracted from *S.* Typhi, Ty2 by hexadecyltrimethylanmonium bromide as described (Szu, Li et al. 1991). Immune response to Vi polysaccharide vaccine is elicited by the production of IgG antibodies and is T cell independent. IgG antibody titres are used for the assessment level of protection against typhoid fever as there are no direct measures of protection for Vi polysaccharide vaccine. IgG antibodies are elicited in 85 to 95% of the vaccinees after the Vi polysaccharide vaccine administration (Plotkin and Bouveret-Le Cam 1995). Protective levels of antibodies are elicited seven days and peak at 28 days post vaccination. The antibody levels to Vi polysaccharide vaccines wane with the passage of time usually after two years. Immune response is boosted if vaccinated after an interval of 3 years but antibodies do not persist for long (Wong, Feeley et al. 1974; Levin, Wong et al. 1975; Robbins and Robbins 1984). Revaccination after 3 years is recommended in the endemic settings and high risk population (travellers, microbiology laboratory technologists) for a sustained protection against typhoid fever (Zhou, Koo et al. 2007).

The Vi polysaccharide vaccine efficacy studies were conducted at large scale in South Africa and Nepal establishing the effectiveness and vaccine immunogenicity. The correlation of Vi antibodies with the Vi polysaccharide vaccine protection as well as the long term persistence of Vi antibodies was documented in the study from South Africa (Acharya, Lowe et al. 1987; Klugman, Koornhof et al. 1996).

The DOMI Programme conducted Vi polysaccharide vaccine effectiveness trails in Karachi, Pakistan, targeting children 2 to 16 years old, and in Kolkata, India, targeting all those who

are 2 years old or older to assess the feasibility of introduction Vi polysaccharide vaccine as a regular public health programme as described in earlier chapters. The protective effectiveness (PE) using clinical protection as endpoint showed that the vaccine protected those who are aged 5-15 years equally from both sites (Kolkata: PE=56% [95%CI: 18%, 77%]; Karachi: PE=58% [95%CI: 8%, 81%]), however, the PE were very different among the pre-school children aged 2-4 years of age (Kolkata: PE=80% [95%CI: 53%, 91%]; Karachi: PE=-38% [95%CI: -192%, 35%]).

The difference in the effectiveness estimates between the studies in Karachi and Kolkata could be due to the difference in study design and the population characteristics. The study in Kolkata included a population older than 2 years including adults, whereas in Karachi, only children were targeted for the vaccination. Therefore the net vaccinated population in Kolkata was much higher than in Karachi (coverage of 60% in Kolkata vs. 22% of total population of the sites). Indirect protection in younger children could have been potentiated by the effect of reduced transmission and hence lower circulation of *S*. Typhi in the environment (Halloran, Haber et al. 1991). The resultant reduced exposure of younger children to *S*. Typhi could have played a major role in reducing the incidence of typhoid fever in the vaccinated clusters of Kolkata. Furthermore, it is worthwhile to note that the dropout rate from the Karachi effectiveness trial was significantly more than what had initially be expected for the sample size calculation, resulting in substantial loss of power to detect the protective effectiveness from the study population. This was not the case in Kolkata site.

For both studies, blood specimens were collected before and after vaccination to examine the immunogenicity after vaccination. Comparison of the immunogenicity data could provide possible immunological differences rather than the epidemiological differences hypothesized for the different estimates on the vaccine effectiveness.

6.2 Methods

6.2.1 Vaccines

The vaccines (Typherix® and Havrix®) were supplied by GlaxoSmithKline Biologicals (GSK), Rixensart, Belgium. Typherix was supplied as 0.5 ml dose of vaccine containing 25 micrograms of the Vi polysaccharide of *S.* Typhi in a clear isotonic colourless solution. A single dose of 0.5 ml is recommended for intramuscular injection in both adults and children two years of age and older. Havrix contained 0.5ml of paediatric dose consisting of but not less than 720 EL.U. of viral antigen, adsorbed on 0.25 mg of aluminium, as aluminium hydroxide. For Kolkata site, adult dose of Havrix (1440 ELU/dose) was also prepared for adult vaccinees. Havrix was supplied as a sterile suspension for intramuscular administration.

6.2.2 Study subjects

A subset of the study population was randomly chosen prior to the vaccination for each site. In Kolkata study, two subjects who had been assigned to each of the two study codes for each cluster to undergo immunogenicity testing. Because of the age-related stratification of the codes, two subjects between the ages of 2 and 18 years and two older subjects were targeted for testing in each cluster. In Karachi, four subjects per cluster were selected.

The selected subjects were marked in the vaccination record books to avoid confusion in the selection of subjects for blood sampling. Identification of the eligible subjects was performed by assessment of the vaccination card distributed prior to the vaccination. The study staff explained the subject or the subject's parent/guardian of the purpose of the blood sampling. History of fever and prior typhoid fever vaccination was ascertained. In case of fever the child was invited for a follow up visit and vaccination and blood collection at a later date. There was no replacement of the subject, such as ones who did not show up, refused, or being ineligible.

6.2.3 Specimen collection and testing

After written informed consent was obtained, 5 ml of blood was collected in sera tubes just before vaccination. The samples at 6 weeks and 2 years were collected at the household

ensuring proper identification. After clotting at room temperature from 30 minutes to 2 hours, blood samples were transported to the clinical laboratory of the National Institute of Cholera and Enteric Diseases (Kolkata study) or of Aga Khan University Hospital (Karachi study). At each respective clinical laboratory, blood samples were centrifuged at 3000 rpm for 15 minutes and the sera was separated aseptically into aliquots within 24 hours after the sampling. Sera were then stored in a freezer at -20°C/-80°C. Each blood sample and aliquot was labelled with pre-marked sticker indicating the blood sampling stage (V1and V2 or V3), the identification number and the initials of the child. The labels did not have any information on the cluster or code of the vaccine. 2 aliquots of the samples were sent to GSK for measurement of Vi antibodies.

Quantitative serum Vi antibody titres (IgG) were measured through enzyme linked immunosorbent assay (ELISA) using the GSK standard (Cordero-Yap, Rivera et al. 2001; Lebacq 2001; Pelser 2001; Ramkissoon and Jugnundan 2001). The assays were processed blindly and the vaccine codes were not broken. The serum analysis was performed at the end of the trial. The serological responses for Vi polysaccharide antibody were defined as percentage of subjects with antibody titre ≥150 ELU/ml (GSK in-house standard). Sero-conversion rate was defined as the percentage of subjects with a 4-fold rise in the antibody titres compared to that of baseline antibody titres. Differences between groups were analyzed for statistical significance by using the chi-square test for comparing proportions and analysis of variance for comparing Geometric Mean Titre (GMT) antibody levels. Logistic regression analysis was performed to assess the association of Vi GMT level at 6 weeks compared to 2 years.

6.2.4 Ethical considerations

The studies were part of the effectiveness trials described earlier. Both studies have been approved by the Institutional Review Board (IRB) of the International Vaccine Institute (IVI), Ethical Review Committee (ERC) of the Aga Khan University, Karachi, Pakistan, IRB of National Institute of Cholera and Enteric Diseases, Kolkata, India and the Indian Council of

Medical Research, Delhi, India. The vaccine manufacturer conducted serologic assays in a blinded manner and played no role in the design of the study and in data analysis.

6.3 Results

6.3.1 Kolkata study

Blood sample was collected from 170, 159 (94%) and 130 (76%) subjects at day 0, week 6 and year two respectively. Only 3 children in the Vi polysaccharide vaccine group and 5 in the Hepatitis A group were below the age of 5 years. 26 children and 22 children were from the school-age group (age 5 to 15 years) for Vi polysaccharide vaccine and Hepatitis A vaccine group, respectively. Majority of the volunteered subjects were adults. There was no statistically significant difference in the number of subjects enrolled between the two groups. 43 subjects (22 in the Vi polysaccharide vaccine group and 21 in the Hepatitis A group) had protective level of antibody prior to vaccination. The mean geometric titre (GMT) for Vi antibodies at baseline prior to the vaccination was 111.4 in the Hepatitis A group and 118.2 ELU in the Vi polysaccharide vaccine group. The baseline GMTs for the two groups were not statistically different (P=0.64). Six weeks after vaccination, the GMT for Hepatitis A group was 107.4 ELU while for Vi polysaccharide vaccine group the GMT was 2505.3 ELU. Two years after vaccination the GMT in the Hepatitis A group was 105.8 and in the Vi polysaccharide vaccine group the GMT was 842.6. At both post-vaccination time points, GMTs in the Vi polysaccharide vaccine group were statistically significantly higher than in Hepatitis A group (6 weeks: P<0.001; 2 years: P<0.001) (Table 6-1).

The subjects achieving protective level of Vi antibodies was statistically significantly higher in Vi group than in Hepatitis A group at 6 weeks after vaccination (74 in Vi group and 14 in Hepatitis A group; comparison of proportion at P<0.001). Similarly, sero-conversion was seen statistically significantly more in Vi group than in Hepatitis A group (71 in Vi group and 3 in Hepatitis A group; comparison of proportion at P<0.001). Protective level of Vi antibodies at

six weeks after vaccination were elicited in 67%, 100%, and 92% of the subjects in the age groups of 2 to less than 5 years, 5 years to less than 15 years, and 15 years or older, respectively. The proportion of subjects having protective level of Vi antibodies or having had sero-conversion at six week was statistically significantly different between Vi polysaccharide vaccine and Hepatitis A vaccine recipients for age groups 5 to less than 15 and 15 and above. Due to the small number of subjects, the age group 2 to less than 5 showed no statistical significance.

The GMT for the vaccinated group fell from 2,505.3 ELU at six week to 842.6 ELU, 2 years after vaccination. The proportion of subjects who attained the protective level of antibody at two years was statistically significantly different in the two groups (55 and 13 in the Vi and Hepatitis A arms; $P<0.001$). This was evident also in age groups 5 to less than 15 and 15 and above for both proportions of those attained the protective level and having had sero-conversion, however, due to the small number of subjects in the age group 2 to less than 5, neither of those serological markers were statistically assessed.

Table 6-1: Results of the Immunogenicity testing from Vi effectiveness trial in Kolkata, India

		Vi group	HAV group	p-value
Overall				
Bleed 1	GMT* (n)	118.23 (79)	111.40 (91)	0.6409
	No.(%) PA**	22(28)	21(23)	0.485
Bleed 2	GMT(n)	2505.28 (78)	107.38 (81)	<.0001
	No.(%)PA	74 (95)	14(17)	<.0001
	No.(%) ≥ 4 fold rise***	71 (91)	3 (4)	<.0001
Bleed 3	GMT(n)	842.64 (63)	105.75 (67)	<.0001
	No.(%)PA	55 (87)	13 (19)	<.0001
	No.(%)≥ 4 fold rise	42 (67)	2 (3)	<.0001
Age<5yrs at baseline				
Bleed 1	GMT(n)	75 (3)	75 (5)	1
	No.(%) PA	0 (0)	0(0)	-
Bleed 2	GMT(n)	1114.1 (2)	75 (3)	0.0005
	No.(%)PA	2 (66.67)	0 (0)	0.035
	No.(%)≥ 4 fold rise	1 (50.0)	0 (0)	0.171
Bleed 3	GMT(n)	75 (1)	75 (2)	1
	No.(%)PA	0 (0)	0 (0)	-
	No.(%)≥ 4 fold rise	0 (0)	0 (0)	-
Age 5-14.9 at baseline				
Bleed 1	GMT(n)	105.34 (26)	92.05 (22)	0.442
	No.(%) PA	6(23)	3(14)	0.363
Bleed 2	GMT(n)	2434.83 (27)	85.91(22)	<.0001
	No.(%)PA	27 (100)	2(9)	<.0001
	No.(%)≥ 4 fold rise	27 (100)	1(5)	<.0001
Bleed 3	GMT(n)	609.69 (22)	86.02 (19)	<.0001
	No.(%)PA	20 (81)	2(11)	<.0001
	No.(%)≥ 4 fold rise	13 (59)	1(5)	0.0002
Age 15 yrs and older at baseline				
Bleed 1	GMT(n)	129.02 (50)	123.24 (63)	0.792
	No.(%) PA	16(32)	18(29)	0.705
Bleed 2	GMT(n)	2630.55 (49)	119.48 (56)	<.0001
	No.(%)PA	45 (92)	12 (21)	<.0001
	No.(%)≥ 4 fold rise	43 (87)	2(4)	<.0001
Bleed 3	GMT(n)	1069.55 (40)	116.91(46)	<.0001
	No.(%)PA	35 (88)	11 (24)	<.0001
	No.(%)≥ 4 fold rise	29 (73)	1(2)	<.0001

Note:

Bleeds: Bleed 1 is from Day 0 (pre-vaccination), Bleed 2 is from Week 6, and Bleed 3 is from Year 2 since vaccination.

*GMT – Geometric Mean Titre

**PA – attaining Protective Antibody level (Vi: ≥150 ELU/ml – GSK in-house standard)

We used the value 75 (midpoint) for those listed as <150 in the report

***Achieved a 4-fold or greater rise in antibodies, in relation to baseline, in the cited bleed

6.3.2 Karachi study

Blood sample was collected from 238, 183 (77%) and 91 (38%) children at day 0, week 6 and year two respectively. 58 children in the Vi polysaccharide vaccine group and 49 in the Hepatitis A group were below the age of 5 years. Three blood samples were drawn from 45 children in the Vi polysaccharide vaccine arm and 36 in the control arm. There was no statistically significant difference in the number of children enrolled between the two groups. Eleven children (5 in the Vi polysaccharide vaccine group and 6 in the Hepatitis A group) had protective levels of antibody prior to vaccination. The mean geometric titre for Vi antibodies at baseline prior to the vaccination was 80.0 ELU in the Hepatitis A group and 78.8 ELU in the Vi polysaccharide vaccine group. The baseline GMTs for the two groups were not statistically different (P=0.60). Six weeks after vaccination the GMT for Hepatitis A group was 85.0 ELU and for Vi polysaccharide vaccine group the GMT was 1,189.1 ELU. Two years after vaccination the GMT in the Hepatitis A group was 91.8 ELU and in the Vi polysaccharide vaccine group the GMT was 166.4 ELU. At both post-vaccination time points, GMTs in the Vi polysaccharide vaccine group were statistically significantly higher than in Hepatitis A group (6 weeks: P<0.001; 2 years: P<0.05) (Table 6-2).

The children achieving protective level of Vi antibodies was statistically significantly higher in Vi group than in Hepatitis A group at 6 week after the vaccination (86 in Vi group and 6 in Hepatitis A group; comparison of proportion at P<0.001). Similarly, sero-conversion was seen statistically significantly more in Vi group than in Hepatitis A group (75 in Vi group and 1 in Hepatitis A group; comparison of proportion at P<0.001). Protective level of Vi antibodies at six weeks after vaccination were elicited in 85% and 100% of the children in the age groups of 2 to less than 5 years and 5 years or older, respectively. The proportion of children having protective level of Vi antibodies or having had sero-conversion at six week was statistically significantly different between Vi polysaccharide vaccine and Hepatitis A vaccine recipients for both age groups.

The GMT for the vaccinated group fell from 1,189.1 ELU at six week to 166.4 ELU, 2 years after vaccination. The proportion of children who attained the protective level of antibody at two years was statistically significantly different in the two groups (13 and 4 in the Vi and

Hepatitis A arms; P<0.05). However, while the proportion of children attaining protective level or having had sero-conversion was statistically significantly higher in those aged above 5 years, neither of those serological markers showed statistical significance in those aged 2 to less than 5 years.

There were 7 children who did not respond to Vi polysaccharide vaccine and did not develop Vi antibodies detectable by ELISA. All non-responders to the Vi polysaccharide vaccine were below the age of 5 years. The Vi antibody levels dropped below the protective level in 25 children after two years. The immune response to Vi capsular polysaccharide in younger children (<5 years) was not statistically different than older children (5 to 16 years) at six weeks and two years after vaccination.

Table 6-2: Results of the Immunogenicity testing from Vi effectiveness trial in Karachi, Pakistan

		Vi group	HAV group	p-value
Overall				
Bleed 1	GMT* (n)	78.78 (122)	80.00 (115)	0.6011
	No.(%) PA**	5(4.10)	6(5.22)	0.6824
Bleed 2	GMT(n)	1189.09 (92)	85.00 (91)	<.0001
	No.(%)PA	86 (93.48)	6(6.59)	<.0001
	No.(%) ≥ 4 fold rise***	75 (81.52)	1 (1.10)	<.0001
Bleed 3	GMT(n)	166.41 (46)	93.89 (45)	0.0421
	No.(%)PA	18 (39.13)	6 (13.33)	0.0052
	No.(%)≥ 4 fold rise	13 (28.26)	4 (8.89)	0.0178
Age<5yrs at baseline				
Bleed 1	GMT(n)	75.00 (57)	76.36 (47)	0.3225
	No.(%) PA	0 (0)	1(2.13)	0.4519
Bleed 2	GMT(n)	820.18 (40)	83.39 (36)	<.0001
	No.(%)PA	34 (85.00)	1 (2.78)	<.0001
	No.(%)≥ 4 fold rise	28 (70.00)	1 (2.78)	<.0001
Bleed 3	GMT(n)	137.33 (20)	99.41 (17)	0.7440
	No.(%)PA	8 (40.00)	2 (11.76)	0.0726
	No.(%)≥ 4 fold rise	5 (25.00)	2 (11.76)	0.4165
Age 5-15.9 at baseline				
Bleed 1	GMT(n)	82.25 (65)	82.63 (68)	0.7716
	No.(%) PA	5 (7.69)	5 (7.35)	1.0000
Bleed 2	GMT(n)	1582.33 (52)	86.06 (55)	0.0002
	No.(%)PA	52 (100.00)	5 (9.09)	<.0001
	No.(%)≥ 4 fold rise	47 (90.38)	0 (0)	<.0001
Bleed 3	GMT(n)	192.90 (26)	90.68 (28)	0.0487
	No.(%)PA	10 (38.46)	4 (14.29)	0.0428
	No.(%)≥ 4 fold rise	8 (30.77)	2 (7.14)	0.0366

Note:

Bleeds: Bleed 1 is from Day 0 (pre-vaccination), Bleed 2 is from Week 6, and Bleed 3 is from Year 2 since vaccination.

*GMT – Geometric Mean Titre

**PA – attaining Protective Antibody level (Vi: ≥150 ELU/ml – GSK in-house standard)

We used the value 75 (midpoint) for those listed as <150 in the report

***Achieved a 4-fold or greater rise in antibodies, in relation to baseline, in the cited bleed

6.3.3 Comparison of immunogenicity testing results from Kolkata and Karachi

Results from Kolkata and Karachi were compared for the comparable age groups: 2 to less than 5 years, 5 years to less than 15 (Kolkata) or 16 (Karachi), and combined 2 to less than 15 (Kolkata) or 16 (Karachi).

At baseline, the GMTs for Vi IgG antibodies were below the protective level for both sites, however, there were statistically significantly higher proportion of children with antibody level higher than the protective level in Kolkata site than in Karachi site (20.7% vs. 4.1%: P<0.01). At six weeks after vaccination, though there is a significant increase in the GMTs in children from both site, the level of GMTs were statistically significantly lower from those in Karachi (2,307.0 ELU vs. 1,189.1 ELU: P<0.01). GMT declined from 6 week to 2 year testing points for both sites and the difference between the two sites was again statistically significantly lower for those in Karachi (556.6 ELU vs. 166.4 ELU: P<0.001). For proportions of those who sero-converted were similar at 6 week and 2 year time points for children from both sites (Table 6-3).

There were only 3 children tested in the age group 2 to less than 5 years for the Kolkata study. Two were tested at 6 weeks after vaccination and only one was available for the test at 2 years. This had led to comparison of the two sites in statistics less meaningful.

For the age group 5 to less than 15 (Kolkata) or 16 (Karachi) years, GMTs were not different between the two sites at 6 week, but it was statistically significantly different at 2 years (609.7 ELU vs. 192.9 ELU: P<0.001). All tested children from both sites had attained protective level at 6 week, but it fell to 90.9% in Kolkata and 38.5% in Karachi at 2 year (P<0.001). Those who sero-converted compared to the baseline were similar between sites at both 6 week and 2 year time points.

Table 6-3: Comparison of the immunogenicity testing results in Vi polysaccharide vaccine groups from Kolkata, India study and Karachi, Pakistan study

		Kolkata	Karachi	p-value
Overall (Age<15yrs in Kolkata and Age<16yrs in Karachi at ZT)				
Bleed 1	GMT* (n)	101.70 (29)	78.78 (122)	0.0095
	No.(%) PA**	6 (20.69)	5 (4.10)	0.0020
Bleed 2	GMT(n)	2307.0 (29)	1189.1 (92)	0.0087
	No.(%)PA	29 (100.00)	86 (93.48)	-
	No.(%) ≥ 4 fold rise***	27 (93.10)	75 (81.52)	0.1459
Bleed 3	GMT(n)	556.61 (23)	166.41 (46)	<.0001
	No.(%)PA	20 (86.96)	18 (39.13)	0.0003
	No.(%)≥ 4 fold rise	12 (52.17)	13 (28.26)	0.0567
Age<5yrs at baseline				
Bleed 1	GMT(n)	75.00 (3)	75.00 (57)	-
	No.(%) PA	0 (0)	0 (0)	-
Bleed 2	GMT(n)	1114.1 (2)	820.18 (40)	0.7891
	No.(%)PA	2 (100.00)	34 (85.00)	-
	No.(%)≥ 4 fold rise	1 (50.00)	28 (70.00)	0.5151
Bleed 3	GMT(n)	75.00 (1)	137.33 (20)	0.0010
	No.(%)PA	0 (0.00)	8 (40.00)	-
	No.(%)≥ 4 fold rise	0 (0.00)	5 (25.00)	-
Age 5 to <15yrs in Kolkata and Age 5 to <16yrs in Karachi at baseline				
Bleed 1	GMT(n)	105.34 (26)	82.25 (65)	0.0609
	No.(%) PA	6 (23.08)	5 (7.69)	0.0479
Bleed 2	GMT(n)	2434.8 (27)	1582.3 (52)	0.1329
	No.(%)PA	27 (100.00)	52 (100.00)	-
	No.(%)≥ 4 fold rise	26 (96.30)	47 (90.38)	0.3170
Bleed 3	GMT(n)	609.70 (22)	192.90 (26)	0.0002
	No.(%)PA	20 (90.91)	10 (38.46)	0.0006
	No.(%)≥ 4 fold rise	12 (54.55)	8 (30.77)	0.7991

Note:

Bleeds: Bleed 1 is from Day 0 (pre-vaccination), Bleed 2 is from Week 6, and Bleed 3 is from Year 2 since vaccination.

*GMT – Geometric Mean Titre

**PA – attaining Protective Antibody level (Vi: ≥150 ELU/ml – GSK in-house standard)

We used the value 75 (midpoint) for those listed as <150 in the report

***Achieved a 4-fold or greater rise in antibodies, in relation to baseline, in the cited bleed

6.4 Discussion

In both study sites, independently, the immune response to the Vi polysaccharide vaccine, given as a single injectable dose, present considerable higher levels of protective antibody levels two years after vaccination. The correlate of protection for typhoid fever vaccine is not well established; however, antibodies to Vi capsular polysaccharide have been used for assessment of Vi polysaccharide vaccine efficacy (Tacket, Ferreccio et al. 1986; Acharya, Lowe et al. 1987; Klugman, Gilbertson et al. 1987; Klugman, Koornhof et al. 1996). Considering the protective titre level set by the GSK in-house standard, most people (ca. 95%) responded to the vaccine at week 6. The difference, however, was seen in the antibody levels in the year 2 sampling between the two sites. In the similar age groups, participants from Kolkata seemed to have responded better compared to the ones from Karachi. The Vi antibody level attained by the Kolkata participants were superior at both week 6 and year 2 samplings, and the protective level was maintained longer. Though anti-Vi IgG GMT level from Karachi participants remain at 166.4 ELU/ml (95% CI: 116.7, 236.3) after 2 years, only 39% of the subjects retained the antibody protective level.

Typhoid fever infection confers natural immunity resulting in the production of Vi antibodies (Siler and Dunham 1939; Gaines, Landy et al. 1961). Differences in prior infection between sites may answer the differences in the response to the vaccine in the young age group; however, only one of the young children aged between 2 and 5 years randomly chosen for immunogenicity testing had protective antibody level before vaccination (0/8 in Kolkata and 1/104 in Karachi). When comparing the older children (5 to 15/16 years of age), there were statistically significantly larger proportion of children having protective level at baseline from those in Kolkata versus those in Karachi ($P<0.05$).

In the study, four and six people did not elicit Vi IgG antibodies after receiving the Vi polysaccharide vaccine in Kolkata and Karachi, respectively. While all four individuals were aged above 16 years in Kolkata, all six were from the youngest age stratum (age 2 to 5 years) in Karachi. These contrasting differences between the two sites have only made the study results more complex to conclude the reasons for the different vaccine impact serologically. It is, however, important to note that Vi polysaccharide vaccine's response in children younger

than five years has been under scientific investigation (Keitel, Bond et al. 1994; Lin, Ho et al. 2001). The association factors such as under-nutrition may have also influenced the response to polysaccharide vaccine in the early life.

The epidemiological differences highlighted in the previous chapter may provide a clue in terms of the differences in the outcome of the effectiveness estimates. In a confined area where there has been a high force of infection, it is possible that many subjects may have been pre-exposed to the organism prior to the vaccination campaign. In theory, if the study clusters are appropriately randomized, there should not be any differences between the two arms, but as disease occur in clusters, it is possible that certain clusters assigned to the control vaccine may have had higher proportion of individuals with pre-exposure. However, this has not been the case for this study, as the proportion of those with protective antibody level is equivalent between two arms. Another hypothesis of the different results may be due to the extremely high incidence of typhoid fever in the control group of the young subjects in Kolkata. When the proportion of individuals with protective titre level is compared between Karachi and Kolkata for the children 5 to 15 years of age, Kolkata subjects had much higher proportion, suggesting that they had previously been exposed at younger age. Observation of the differences may provide many possible epidemiological explanations; however, these do not explain the results of lower immunological responses in Karachi subjects compared to the Kolkata subjects.

6.4.1 Potential limitations

Since Kolkata study included all ages above 2 years to the study, immunogenicity candidates were selected from two strata: under and above 18 years of age. This has led to a very minimal number of test subjects in less than 5 years of age. There were only 3 children tested in the age group 2 to less than 5 years. Furthermore, among those three subjects, two were tested at 6 weeks after vaccination and only one was available for the test at 2 years. This small number had led to comparison of the immunogenicity results from two sites statistically less meaningful.

The assessment of the Vi antibodies is based on an ELISA technique of the GSK Biologicals, using GSK Biologicals for internal use. The cut-off has been standardized following appropriate procedures (Cordero-Yap, Rivera et al. 2001; Lebacq 2001; Pelser 2001; Ramkissoon and Jugnundan 2001). However, this limits the ability to compare the results from the study with studies of immune response conducted elsewhere.

6.4.2 Implications

Typhoid fever incidence is higher in children younger than five years of age compared to older children in the endemic regions of the world. The immune response elicited by natural infection as well as by the vaccine in the younger children less than five years needs further exploration. In the absence of an effective vaccine for younger children other methods of prevention at household, such as hand washing, and drinking clean water should be adopted to prevent typhoid fever in children less than five years (Luby, Faizan et al. 1998; Gasem, Dolmans et al. 2001).

Typhoid fever incidence continues to be high in children and in the absence of long term interventions such as water and sanitation, vaccination is an alternate short term strategy. However, Vi polysaccharide vaccine conferred conflicting level of Vi IgG antibodies in children younger than five years between the two sites, and hence the true protection may be dependent on multiple factors that still need further investigation. The duration of protection for older children and adults is three to five years. This will require revaccination every three to five years if vaccination programme is introduced. Such limitations affect advocating the programmatic use of Vi polysaccharide vaccine in young children. Conjugation of polysaccharide vaccines to proteins has resulted in a better immunogenicity to polysaccharide vaccines at all ages (Dintzis 1992; Cadoz 1998; Goldblatt 1998). Vi-rEPA conjugate vaccine has also shown promising results in randomized trials in Vietnam (Kossaczka, Bystricky et al. 1997; Lin, Ho et al. 2001; Mai, Phan et al. 2003; Canh, Lin et al. 2004). Such protein conjugate Vi polysaccharide vaccine that provides long term protection and confers immunity

in infants and toddlers will have a significant impact on incidence of typhoid fever in endemic countries such as India and Pakistan.

7 CONCLUSION

7.1 Summary

Prior to conducting effectiveness trials for the DOMI Programme, disease surveillance systems were set up to assess typhoid disease burden in five Asian countries (Ochiai, Acosta et al. 2008). A census was conducted in each site to accurately obtain the population database. These study populations were followed up for a year through fever surveillance at health centres in the areas. Surveillance included both systematic collection of clinical information recorded on standardized forms, and collection of a single blood culture by venipuncture during febrile episodes. Blood culture bottles were transported to the laboratory the same day of the sampling, incubated, and observed for growth. Specimens identified as S. Typhi were subjected to antibiotic susceptibility testing using Kirby Bauer disc diffusion method.

In the five Asian sites, a total of 441,435 persons in the targeted age groups were under surveillance for one year. The incidence rate of typhoid fever varied widely among sites (15.3 cases per 100,000 persons 5 to 60 years old in China to 451.7 cases per 100,000 persons 2 to 15 years old in Pakistan), and even within the 5 to 15 year old age group, which was under surveillance in all the study sites (24.2 cases per 100,000 persons per year in Vietnam to 493.5 cases per 100,000 persons per year in India). For sites where surveillance was additionally undertaken in pre-school children (2 to 5 years of age), the incidence in these younger children was found to be comparable to that for school aged children and adolescents (573.2, 340.1, and 148.7 per 100,000 children in Pakistan, India, and Indonesia, respectively). These findings confirm the traditional notion that typhoid is primarily a problem for school aged children and adolescents in endemic settings, and that at least in South Asia pre-school children are at equally high risk.

Antimicrobial susceptibility also varied by site. Nearly 60% of the 127 tested Pakistani *S.* Typhi isolates were resistant to chloramphenicol, ampicillin, TMP-SMX, and nalidixic acid. In contrast, all 15 tested Chinese *S.* Typhi isolates were susceptible to all antimicrobials. MDR

was observed from 85/127 (67%) *S.* Typhi isolates from Pakistan, 4/18 (22%) from Vietnam, and 8/113 (7%) from India. Nalidixic acid resistance was found in 75/127 (59%) *S.* Typhi isolates from Pakistan, 65/113 (58%) isolates from India and 8/18 (44%) isolates in Vietnam, but was not detected in China or Indonesia. One *S.* Typhi isolate (0.9%), from India, was resistant to ciprofloxacin. One *S.* Typhi isolate (0.8%) from Indonesia was found to be resistant to third-generation cephalosporins (cephalothin).

The high burden of the disease in South Asia supported the need to examine the vaccine effectiveness of the currently available vaccines. Vi polysaccharide typhoid vaccine was considered more suitable for developing country settings considering its single dose regimen and its better temperature stability data. In order to estimate the impact of the vaccine when used under public health conditions, cluster randomized effectiveness trials with typhoid Vi polysaccharide vaccine were designed and conducted in Kolkata and in Karachi.

The findings of the cluster-randomized effectiveness trial in Kolkata indicated that Vi vaccine conferred 61% protection against typhoid fever in a general population in Kolkata, and was associated with minimal side-effects. There were no previous trials evaluating clinical protection against typhoid fever in pre-school children, an age group now appreciated to be at high risk of typhoid fever, especially in urban areas of South Asia. The trial in Kolkata, for the first time, found clinical protection in children vaccinated before the age of five years (PE=80%). Additionally, the trial demonstrated significant herd protective effects, as demonstrated by indirect protection of non-vaccinees by Vi vaccination (PE=45%).

The use of Vi polysaccharide vaccine in Karachi, Pakistan also resulted in significant protection (PE=57%) for children 5 to 16 years of age against typhoid fever and was associated with minimal or no vaccine related adverse events. However, no protection was observed in the younger age group (2 to 5 years of age) and we did not find any statistically significant indirect protection for the study population.

The difference in the effectiveness estimates in younger children between the trials in Kolkata and Karachi may be due to the difference in study design and the population characteristics. The study in Kolkata included a population older than 2 years including adults, whereas in

Karachi, only children were targeted for the vaccination. Therefore the net vaccinated population in Kolkata was much higher than in Karachi (coverage of 60% in Kolkata vs. 22% of total population in Karachi sites). Indirect protection in younger children could have been potentiated by the effect of reduced transmission and hence lower circulation of *S*. Typhi in the environment.

Immunogenicity studies were undertaken for randomly selected persons from both of these sites. Five millilitres of blood was collected in sera tubes just before vaccination. The samples at 6 weeks and 2 years were collected at the household ensuring proper identification. Two aliquots of the sera samples were sent to GlaxoSmithKline (GSK) Biologicals to measure quantitative serum Vi antibody titres (IgG) through enzyme linked immunosorbent assay (ELISA) using the GSK standard. The serological responses for Vi polysaccharide antibody were defined as percentage of subjects with antibody titre ≥150 ELU/ml (GSK in-house standard). Sero-conversion rate was defined as the percentage of subjects with a 4-fold rise in the antibody titres compared to that of baseline antibody titres.

At baseline, the Geometric Mean Titres (GMTs) for Vi IgG antibodies were below the protective level for both sites, however, there were statistically significantly higher proportion of children with antibody level higher than the protective level in Kolkata site than in Karachi site (20.7% vs. 4.1%). At six weeks after vaccination, though there is a significant increase in the GMTs in children from both site, the level of GMTs were statistically significantly lower from those in Karachi (2,307.0 ELU vs. 1,189.1 ELU). GMT declined from 6 week to 2 year testing points for both sites and the difference between the two sites was again statistically significantly lower for those in Karachi (556.6 ELU vs. 166.4 ELU). The proportions of those who sero-converted were similar at 6 week and 2 year time points for children from both sites.

In these serological assessments, four people in Kolkata and six people in Karachi did not elicit Vi IgG antibodies after receiving the Vi vaccine. While all four individuals were aged above 16 years in Kolkata, all six were from the youngest age stratum (age 2 to 5 years) in Karachi. These contrasting differences between the two sites have only made the study results more complex to conclude the reasons for the different vaccine impact serologically.

The association factors such as under-nutrition may have influenced the response to polysaccharide vaccine in the early life.

The data from these effectiveness trials gave a conclusive evidence of the protection conferred by the Vi polysaccharide vaccine in at least children older than 5 years of age. As an effectiveness trial, where an objective is to measure the impact in real-life settings, participants were not as strictly controlled (e.g. uptake of medicine, use of other vaccines, etc.). Even in such settings, the vaccine's protective effectiveness of approximately 60% after 2 years is broadly similar to the individually randomized licensure trials conducted in South Africa, Nepal, and China. Targeted vaccination programme in high endemic areas, as stipulated in the WHO Position Paper, suggest the potential for effective control of typhoid fever in places like India and Pakistan with the Vi polysaccharide vaccine targeting school-aged children.

7.2 Discussion

In addition to the studies presented in this thesis, recent field studies through the DOMI programme have provided new data on the magnitude of typhoid disease, especially in Asia, including incidence rates, age distribution patterns and trends in antibiotic resistance of S. Typhi, as well as on the economic burden of the disease (Bahl, Sinha et al. 2004; Poulos, Bahl et al. 2004; Do, Whittington et al. 2006; Chen, Stanton et al. 2007; Cook, Jeuland et al. 2008; Kelly-Hope, Alonso et al. 2008; Memon, Pach et al. 2008; Ochiai, Acosta et al. 2008; Sur, Manna et al. 2009). Large vaccine demonstration studies in typhoid-endemic countries of Asia have yielded data on the effectiveness of Vi polysaccharide vaccine; the feasibility, acceptability and costs of large-scale community- or school-based vaccination; and the population demand for new-generation typhoid vaccines (Yang, Acosta et al. 2005; Agtini, Ochiai et al. 2006; Chen, Stanton et al. 2006; Khan, Ochiai et al. 2006; Thiem, Danovaro-Holliday et al. 2006).

The production capacity, supply and prices of the Vi polysaccharide vaccine have also increased considerably in the last decade. In addition to the international pharmaceutical

producers, GlaxoSmithKline Biologicals and sanofipasteur, there is a growing number of manufacturers in developing countries that produce Vi polysaccharide vaccines, such as Bharat Biotech in India, DAVAC in Vietnam, Finlay Institute in Cuba, several National Biological Institutes in China, and this number is likely to grow larger in the near future. These developments have greatly increased the feasibility and affordability of broader adoption of typhoid vaccination in high-risk areas of endemic countries.

In China, where the Vi polysaccharide vaccine has been introduced in high-risk areas and food handlers, the incidence of typhoid fever has reduced to very low level (DeRoeck, Ochiai et al. 2008). The typhoid Vi polysaccharide vaccine has been produced locally since 1996 in China. The vaccine was licensed in China based on a clinical trial, which found efficacy to be 69% at 19 months (Yang, Wu et al. 2001). School-based typhoid vaccination programs have been common across the high-incidence provinces. These programs require coordination between the local CDCs and the schools where vaccination takes place. School nurses play an important role in implementing the programme. Parents were informed of the typhoid vaccination programs and required to pay for the vaccines. These programs were used routinely across Guangxi and Hunan provinces. Vaccination of food handlers was uniformly encouraged in some typhoid endemic provinces including Guangxi, Sichuan, Guizhou, and Yunnan. Vaccination is encouraged to reduce spread of typhoid from infected food handlers to those purchasing food. Vi vaccination has also been used to control outbreaks in Guangxi, Sichuan, and Guizhou provinces. Outbreaks are confirmed when more than five enteric fever cases are observed within one week within a unit and when at least one case is laboratory-confirmed as typhoid. During outbreak responses, vaccines are often encouraged for the entire populations of affected units.

These targeted approaches in China have reduced the incidence of typhoid substantially to the point that many of these provinces now conduct vaccination only in very limited fashion. From Guangxi province, where detailed data is available, more than 4.3 million doses of Vi polysaccharide vaccine have been used between 1996 and 2009 to control the disease (Figure 7-1). More than 90% of the 4.3 million doses used were consumed in the high-risk

areas, where approximately 78% of the doses were delivered to school children, with the remaining 22% delivered to food workers.

The Vi vaccination programme in Vietnam targeted children in the high-risk areas since 1997 (DeRoeck, Ochiai et al. 2008). Vi polysaccharide vaccine was initially procured from Sanofi Aventis, until local manufacturer began producing in 1999. Selection of the districts for vaccination is based on typhoid disease reports provided by each province during quarterly regional epidemiological meetings. National EPI, located within the National Institute of Hygiene and Epidemiology (NIHE), considered the budget and the risk levels prior to providing the vaccines to the selected districts. The first year of the programme in a new district involves “catch-up” vaccination campaigns of 3-10 year olds, followed by vaccination of 3 or 4 year olds only in subsequent years. Between 2001 and 2006, more than four million 3-10 year olds have received the vaccine (Figure 7-2).

Figure 7-1: Vaccine Coverage and Typhoid Incidence in Guilin, Guangxi Province, China

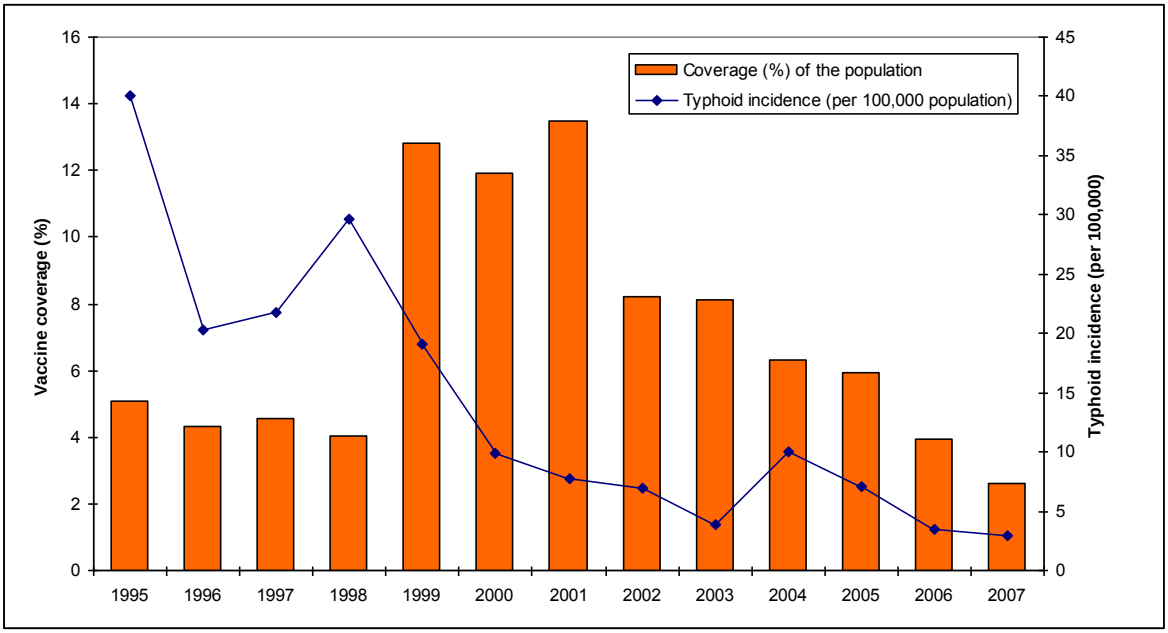
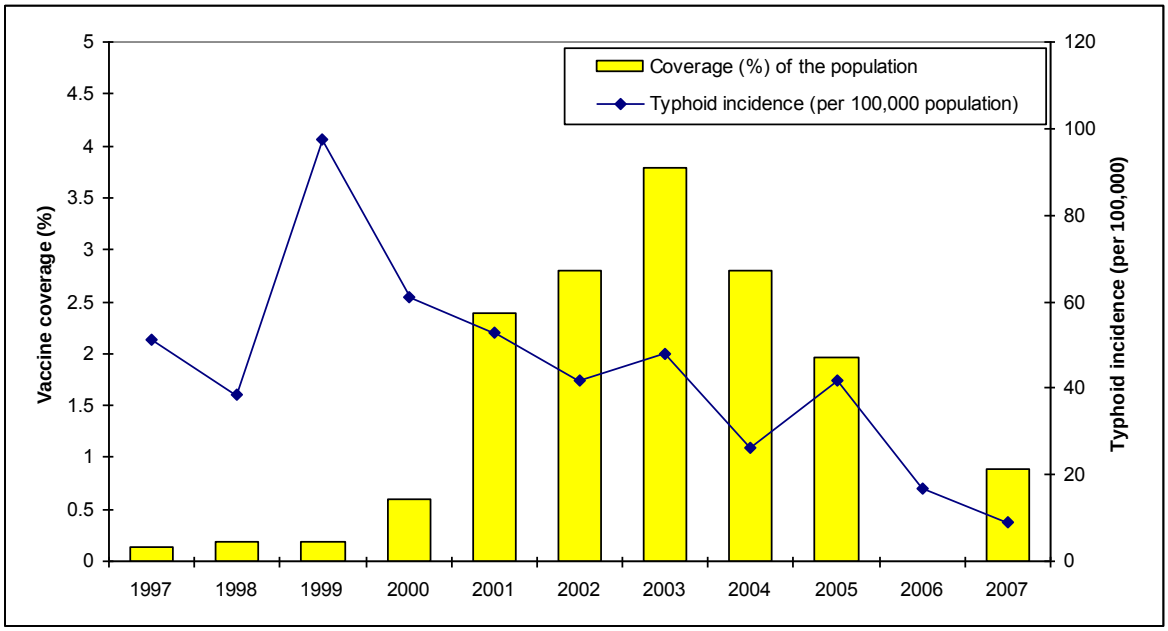


Figure 7-2: Vaccine Coverage and Typhoid Incidence in North-western Region of Vietnam



In addition to China and Vietnam, the State of Delhi in India has begun organizing its own vaccination programme since 2004 (DeRoeck, Ochiai et al. 2008). The programme targets pre-school children which were shown to be at high risk of getting the disease in a previous study (Sinha, Sazawal et al. 1999). Vaccines for their EPI programme (UIP; Universal Immunization Programme) are procured centrally from Federal budget, but as typhoid vaccine is not listed in the UIP, the state government of Delhi has been procuring Vi polysaccharide vaccine directly from local manufacturer at public sector pricing. The vaccine is delivered through the public health system targeting children 2 to 4 years of age. More than 1 million doses have been given to the eligible children through this programme; however, there have been no formal assessment of impact was done.

The Vi polysaccharide vaccine has also been used in certain special situations. As a preventive action following the Tsunami in 2004, Pondicherry local government had used 17,000 doses of Vi polysaccharide vaccine to general population (Sengupta 2005). As a response to an outbreak, a Pacific island nation of Fiji has used the Vi polysaccharide vaccine to control the outbreak and prevent further spread from the neighbouring villages in 2010, following the cyclone Tomas (TVNZ 2010). The government of Sri Lanka has set up a guideline in 2009 to vaccinate the internally displaced population after the civil war (Epidemiology Unit 2010).

Despite the slow progress, some countries and local governments have begun to consider typhoid vaccine for their public health programme. In a pilot fashion, the VIVA Initiative by the IVI will conduct school-based vaccination in Karachi, Pakistan and Kathmandu, Nepal, targeting 200,000 and 120,000 students, respectively. In addition to the outbreak response in Fiji, the government is now considering to introduce a routine immunization programme through their school health programme. Sri Lanka is about to begin a large-scale Vi immunization programme in high risk districts. And in Africa, Kenya has begun a small-scale typhoid immunization programme targeting food handlers at boarding schools.

These developments go in parallel with the review of recent data. In 2007, the Strategic Advisory Group of Experts (SAGE) at the WHO had reviewed the typhoid and typhoid vaccine data and published a revised, strengthened recommendation on the use of the vaccine (World

Health Organization 2008). Considering the heterogeneity of disease burden and antibiotics resistance at inter- and intra- country level, the WHO Position Paper on typhoid vaccine suggests the use of typhoid vaccines in high-risk population and in high endemic areas (World Health Organization 2008). Typhoid endemic countries are urged to review their disease burden data to identify the high-risk age groups and to introduce the vaccination programme in conjunction with other measures, such as improvement in water and sanitation, provision of health education, and training of healthcare workers for proper treatment and diagnosis.

The data from the effectiveness trials in Kolkata, India and Karachi, Pakistan, as presented in this thesis, gave a conclusive evidence of the protection conferred by the Vi polysaccharide vaccine in at least children older than 5 years of age. As an effectiveness trial, where an objective is to measure the impact in real-life settings, participants were not as strictly controlled (e.g. uptake of medicine, use of other vaccines, etc.). Even in such settings, the vaccine's protective effectiveness of approximately 60% after 2 years is nearly identical to the individually randomized licensure trials conducted in South Africa, Nepal, and China. Targeted vaccination programme in high endemic areas, as stipulated in the WHO Position Paper, and evidences of impact from such use in China and Vietnam suggest the potential for effective control of typhoid fever in places like India and Pakistan.

However, the two effectiveness trials could not produce similar results in younger children between 2 and 4 years of age. The difference in the effectiveness estimates between the studies in Karachi and Kolkata could be due to the difference in study design and the population characteristics. The study in Kolkata included a population older than 2 years including adults, whereas in Karachi, only children were targeted for the vaccination. Therefore the net vaccinated population in Kolkata was much higher than in Karachi and may have resulted in different forces of infections in the two communities. Furthermore, there was a distinct lower development of immunity from Karachi participants. This outcome was unexpected as previous immunogenicity studies with Vi polysaccharide vaccine have been consistent. All possible programmatic irregularities were reviewed (e.g. injection practice, cold chain, case identification, etc.), but there was no clue to suggest the outcome. There are

other possible elements, such as nutritional level, prior exposure, etc., but the study did not look into such details and there was no information or data to prove such interaction.

Another major finding, yet confirmed from only one site was the indirect protection. In Kolkata site, unvaccinated neighbours of the vaccination clusters were protected by 44% despite the mere 60% coverage of the community. In Karachi, no indirect protection was observed. This was potentially due to the fact that the vaccination covered only children aged 2 to 16 years of age covering only approximately 22% of the community. Modelling of total and indirect protection may not be simple for disease like typhoid fever. There are many factors beyond vaccine uptake and characteristics such as human interactions, population density, eating and sanitation practices, climate, and water system that need to be considered to make a model that can suggest levels of Vi polysaccharide vaccine coverage required for indirect protection. Nonetheless, *a priori* preparation of analytical plans for both sites authenticates the results and that we know now at a place and condition similar to the site in Kolkata would induce indirect protection at 60% coverage.

Despite the progress in new vaccine development, there are still many vaccines that are still not being used by the people most affected. Vaccines against typhoid, cholera, Japanese encephalitis, meningococcal meningitis are such example. Typhoid fever incidence continues to be high in children, and in the absence of long term interventions such as water and sanitation in many parts of the developing world, vaccination may be an alternate short to intermediate term strategy. Vi polysaccharide vaccine has been available in the market for about 20 years, yet, it is still considered a traveller's vaccine. The price of the vaccine should not be considered as a burden and now there are enough evidences that the vaccine should be introduced in a targeted fashion. However, it is important to note that Vi polysaccharide vaccine conferred contrasting clinical protection results and different level of Vi IgG antibodies in children younger than five years between the two effectiveness trial sites, and hence the true protection may be dependent on multiple factors that still need further investigation. The duration of protection for older children and adults is three to five years. This will require revaccination every three to five years when vaccination programme is introduced. Such limitations affect advocating the programmatic use of Vi polysaccharide vaccine in young

children. Conjugation of polysaccharide vaccines to proteins has resulted in a better immunogenicity to polysaccharide vaccines to all ages (Dintzis 1992; Cadoz 1998; Goldblatt 1998). Such protein conjugate Vi polysaccharide vaccine that provides long term protection and confers immunity in infants and toddlers will have a significant impact on incidence of typhoid fever in endemic countries.

7.3 Future direction

While the presented studies provided concrete evidence of Vi polysaccharide vaccine's protection in school-aged children against typhoid fever, there was no clear evidence to why the vaccine performed very well in Kolkata, but not at all in Karachi for younger children aged between 2 and 5 years. It may be scientifically worthwhile to look into the matter; however, for the purpose of public health, the currently available Vi polysaccharide vaccine shall be introduced in school-based program in high burden area, while continuing the development of vaccines that works in younger children, such as conjugated vaccines. Nonetheless, the actual introduction of the vaccine will depend upon the cost-effectiveness of the vaccination program with whichever the vaccine to be used; hence it is also essential to look into economic aspects of the vaccine and vaccination programs for typhoid fever prevention.

Supposedly that a country decides to introduce the vaccine in its public health program, establishing the disease surveillance system will be important in order to assess the impact of vaccine introduction. It is essential to work with local governments to strengthen the febrile illness surveillance system and assist in equipping the laboratories with blood culture facilities. Countries which have the mandatory reporting system will need to ensure every single typhoid case, confirmed or suspected, reported to the central government to evaluate the secular trend of the disease prevalence throughout the vaccine introduction period and beyond. If such surveillance system can include beyond the vaccine-targeted age group, then the system can potentially evaluate the possible presence of herd protection to other age groups.

Finally, it is important to address the limited knowledge in the roles of carriers in typhoid fever transmission. It is known that typhoid carriers are likely the source of infections and outbreaks in many parts of the world. Yet there are very few contemporary data on prevalence and their roles in transmission. Studying the carriers and their roles in the community will assist developing a better typhoid control program, which will not only include the vaccination as a component, but also water and sanitation, as well as carrier identification and treatment.

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International Vaccine Institute
Diseases of the Most Impoverished (DOMI)
Typhoid Program

Analytical Plan of the Vi Effectiveness Trials Kolkata, India

January/2008

1. Overview

1.1 Corresponding protocol and its identification:

Randomized controlled evaluation of protection by the Vi polysaccharide vaccine against typhoid fever in eastern Kolkata, West Bengal, India (NCT00125008)

The study has been registered with the US clinical trial registry (http://www.clinicaltrials.gov) which is mandated for publication of clinical trial results. Registration number is given in the parentheses.

1.2 Investigational product:

Vi capsular polysaccharide vaccine

1.3 Indication:

Prevention of infection by *Salmonella* Typhi

1.4 Sponsor:

The Disease of the Most Impoverished Program – Funded by the Bill and Melinda Gates Foundation and administered by the International Vaccine Institute

The DOMI Program
International Vaccine Institute
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1.5 Study phase:

Phase 3b Effectiveness evaluation (multi-site)

1.6 Principal Investigators:

Sujit K Bhattacharya – Indian Council of Medical Research (National Institute of Cholera and Enteric Diseases)
John D Clemens – International Vaccine Institute

1.7 Co-Investigators:

Dipika Sur – National Institute of Cholera and Enteric Diseases
Byomkesh Manna – National Institute of Cholera and Enteric Diseases
R Leon Ochiai – International Vaccine Institute
Mohammad Ali – International Vaccine Institute

1.8 Summary:

This document describes the specific procedures to be followed in the statistical analysis of the above-mentioned trial and the format of data output. The primary features of the statistical analysis plan were specified in the original protocol. This document focuses on the statistical issues related to the primary efficacy analysis.

2. Study Synopsis

TITLE	**Randomized controlled evaluation of protection by the Vi polysaccharide vaccine against typhoid fever in eastern Kolkata, West Bengal, India**
STUDY OBJECTIVES	**Primary** 1. To determine total protection conferred by Vi polysaccharide vaccine, given in a 1-dose schedule, against episodes of blood-culture confirmed *S. typhi* **Secondary:** 2. To determine total protection conferred by Vi against episodes of clinical typhoid fever 3. To determine total protection conferred by Vi against episodes of persistent fever 4. To determine indirect protection conferred by Vi against episodes of blood culture-confirmed *S. typhi,* clinical typhoid fever, and persistent fever 5. To determine overall protection conferred by Vi against episodes of blood culture-confirmed *S. typhi,* clinical typhoid fever, and persistent fever 6. To evaluate the safety and immunogenicity of Vi vaccine following administration through a mass vaccination campaign. 7. To estimate the logistic feasibility of a mass immunization campaign, 8. To evaluate knowledge, attitudes, beliefs and practices among the residents and health providers in the study area regarding Vi vaccination and typhoid, and 9. To estimate the cost-effectiveness of preventing typhoid fever.with Vi vaccine
TRIAL LOCATION	Ward 29 and 30, Kolkata, West Bengal, India
STUDY PERIOD	May 2003-Nov 2004 Pilot Phase (19 months) approved previously by WHO and local ethical committee Nov 2004-Dec 2006 Vaccination Phase (26 months)
STUDY DESIGN	Evaluation-blinded cluster-randomized control effectiveness trial
STUDY SUBJECTS	Inclusion criteria • Permanent residents, registered in the project censuses • Provide informed consent to receive vaccines • Age: ≥ 2 years Exclusion criteria • Fever (>37.5° C, axillary) • Pregnancy • Lactating
SAMPLE SIZE	Approximately 41,514 individuals will be needed in order to have 80% power to detect a 60% vaccine protection at a 5% level (two-sided), assuming a loss to follow-up of 10% per year. Each cluster will be composed of groups of premises. Cluster size will be 600-700 individuals (80 clusters). Expected cumulative incidence rate of blood culture positive S typhi in the placebo group: 2/1000.

STUDY VACCINES	***Investigational product:*** **Vi polysaccharide typhoid vaccine**, Typherix ® (GlaxoSmithKline) **Form**: Clear liquid in a 1 dose pre-filled syringe **Dose**: single 0.5ml dose (25ug of the purified Vi polysaccharide of *S.* typhi) for both children and adults **Route**: IM in the deltoid area ***Active Control:*** **Hepatitis A vaccine:** Havrix ® (GlaxoSmithKline) **Form**: Suspension liquid in a 1 dose pre-filled syringe. **Dose**: pediatric→single 0.5ml dose (720 EL.U of viral antigen); adult→ single 1ml dose (1440 EL.U of viral antigen). **Route**: IM in the deltoid area
CODES	B, U, L, X – two codes were for children (defined as 2 to ≤ 18 years of age) and two codes for adults Investigators were aware of the pediatric vs. adult codes, but not of the agent within each age group
EVALUATION CRITERIA	<u>Effectiveness</u> Total, indirect, and overall protection by Vi polysaccharide vaccine against: • Episodes of fever in which *S.* typhi is isolated from blood culture. • Episodes of fever which meets the definition of clinical typhoid • Episodes of persistent fever <u>Safety</u> • Local and systemic events detected in first 3 days after immunization.via active surveillance (subset of 320 subjects) • Clinical events detected by passive surveillance in the first month after vaccination (entire study population) • Deaths occurring in the first month following vaccination (entire study population) <u>Vi Immunogenicity (N: 320, same subset as above)</u> • Levels of serum IgG anti-Vi antibody titers (GMT) • Seroconversion rate (defined as greater than or equal to 4-fold rise of serum antibodies in bleed 2 relative to bleed 1) • Seroprotection rate (defined as attainment of a titer of serum antibodies of greater than or equal to 1µg/ml) <u>Vaccine logistics, costs and feasibility</u> • Resources, including personnel, needed for vaccine procurement, storage, transport and vaccine delivery, • Efficiency of vaccine storage, transport, and handling • Safe vaccination practices including vaccine administration and disposal of sharps • Detailed costing of vaccine delivery (project and vaccination level) • Vaccination costs (public and private)
DURATION OF STUDY PERIOD (per subject)	2 years after vaccination

3. *A priori* definitions

Cluster: The study was a cluster randomized trial. The unit of the randomization was a *cluster*. A cluster was a collection of geographically contiguous households within an area with clearly defined boundaries. The study site was divided into 80 such clusters of similar population sizes.

Zero time: We defined the beginning of the study period as *zero time*. The *zero time* is defined separately for the vaccinees and non-vaccinees. For vaccinees, the *zero time* is the date of vaccination; and for non-vaccinees, it is the median date of vaccination of the persons vaccinated in the cluster.

Age- and residence- eligibility for the trial: Persons present at the time of vaccination, as reflected in the pre-vaccination census, and aged 2.0 years and above at the *zero time* were eligible to receive the vaccine.

Medical exclusion criteria for the trial: Individuals with one or more medical contraindications to either of the vaccines at *zero* time were excluded. These contraindications included acute fever, lactation and pregnancy.

Absentees: Absentees were people known to reside in the field area during vaccination who failed to be contacted by teams recruiting subjects for vaccination. The vaccination period was approximately 5 weeks long. These subjects either were in the pre-vaccination census or were noted retrospectively in the close-out census to have been residing in the study area at the time of vaccination.

Refusers: The trial required signed informed consent for participation. Persons who refused to provide informed consent for participation (or children whose parents refused participation) were considered refusers.

Censuses: Four censuses were carried out to collect the household information at different time points. These censuses captured the *de jure* population (defined as persons who stated that their residence in the study area was their customary residence) and recorded the demographic events of the population in the study area. Two censuses were conducted prior to the vaccination, one at the onset of pre-vaccination surveillance period and the other just before vaccination. Two censuses were conducted after vaccination during the two-year follow-up period to update the study area population through vital demographic events. The final census was termed the close-out census.

Vital demographic events for the analysis:

> **In-migrants:** Individuals who were not residents at *zero time* but who were identified in the final two censuses as having moved into the study area were termed in-migrants.
>
> **Newborns:** Individuals who were not residents at *zero time* but who were identified in the final two censuses as having been born in the study area were termed newborns.
>
> **Out-migrants:** Individuals who were residents of the study area at *zero time*, but who were identified in the final two censuses as having moved out of the study area, were termed out-migrants.
>
> **Internal-migrants:** Individuals who were residents of the study area at *zero time*, but who were identified in the final two censuses as having changed residences in the study area, were termed internal-migrants.

Randomized population: Individuals who were known to be present at *zero time*, either by being in the pre-vaccination census or by being retrospectively identified as having been present at *zero time* in the post-vaccination censuses, were considered randomized according to the assignment of the cluster of residence at *zero time*.

Vaccinee: A member of randomized population who received one of the two study vaccines.

Loss to follow up: Those who either died or moved out from the study area during the follow up period.

Strata for randomization: Blocked randomization to study agents was done after stratifying the clusters into 4 categories based on information in the pre-vaccination census. The strata considered the number of adults and children (≤18 years of age at zero time) in the cluster separately. The strata were 1) <500 adults and <200 children; 2) <500 adults and ≥200 children; 3) ≥500 adults and <200 children; and 4) ≥500 adults and ≥200 children.

Pre-vaccination period: Pre-vaccination period is the period before the *zero time*.

Study follow-up period: Follow-up for typhoid fever, clinical typhoid fever, and paratyphoid fever commenced from the day after the *zero time*. Follow-up for out-migration and death began at *zero time*. The maximum duration of follow-up was 2 years (730 days) after *zero time*, which was considered the termination date of surveillance.

Post-follow-up period: The time after the completion of follow-up period is defined as post-follow-up period. Onset of events occurring in this period will not be considered for analyses.

Objective Fever: An axillary temperature ≥38°C (100.4°F).

Subjective Fever: Historical recall of having fever

Persistent fever visit: A report by a patient of having had at least 3 days of fever at the time of presentation to the treatment center

Febrile episode: Visits for subjective or objective fever were concatenated into single episode if the recalled date of onset of fever for one visit was within 14 days of the date of presentation from the previous visit. The date of onset is taken as the recalled onset of fever for the first component visit of the episode.

Persistent fever episode: Febrile episodes with at least one component visit with objective fever were considered persistent if the duration between the onset of first component visit and the date of presentation for the last component visit was at least 3 days. The date of onset is taken as the recalled onset of fever for the first component visit of the episode.

Blood culture proven typhoid fever: A subject was considered to have blood culture proven typhoid fever if at least one blood culture yielded a growth of *S.* typhi. Such cases were considered identity-confirmed if a visit to home conducted after the positive blood culture confirmed that the subject whose name was provided in the treatment center in fact visited the treatment center on the day of blood culture.

Typhoid fever episode: A febrile episode with blood culture proven typhoid fever was defined as typhoid fever episode. The date of onset is taken as the recalled onset of fever for the first component visit of the episode.

Blood culture proven paratyphoid fever: A subject was considered to have blood culture proven paratyphoid fever if at least one blood culture yielded a growth of *S.* paratyphi A or B. Such cases were considered identity-confirmed if a visit to home conducted after the positive blood culture confirmed that the subject whose name was provided in the treatment center in fact visited the treatment center on the day of blood culture.

Paratyphoid fever episode: A febrile episode that is not typhoid fever episode, but has at least one blood culture positive for *S. paratyphi* A or B was defined as paratyphoid fever episode. The date of onset is taken as the recalled onset of fever for the first component visit of the episode.

Clinical typhoid fever episode: A febrile episode that is not typhoid or paratyphoid fever episode but met *a priori* clinical and serological criteria for clinical typhoid fever. The date of onset is taken as the recalled onset of fever for the first component visit of the episode.

4. Validation of blood culture isolates

Blood culture isolates of *S.* typhi and *S.* paratyphi identified by NICED laboratories during the study period have been checked for their identities at a reference lab. The majority of *S.* typhi and *S.* paratyphi isolates were confirmed at the Wellcome Trust Research Unit in Ho Chi Minh City, Vietnam, and the remaining portion were tested at the IVI lab.

Comparing the results from WTRU/IVI lab and IVI database for the follow up period

	Ref lab ST	**Ref lab SPA**	**Ref lab NTS**	**Ref lab not tested**
NICED ST	182	7	1*	1**
NICED SPA	2	130	0	2***
NICED NTS	0	0	2	0

ST- *S. typhi;* SPA- *S. paratyphi*; NTS – Other Salmonella
NICED- Isolate identification by NICED; Ref Lab—Isolate identification by the reference laboratory
* Noted "atypical *S.* typhi" in the NICED results
** Specimen did not grow
*** 1 Specimen did not grow, 1 Specimen not available

The proportion of discordant results was minimal and within accepted value (John Wain, personal communication). We accepted the results from the reference laboratories, except for the 3 specimens which were not tested at the reference laboratories, for which we accepted the original results from NICED.

5. Strategy for arriving at a definition of clinical typhoid fever

To arrive at a definition of clinical typhoid fever, all persistent (≥ 3 day pre-presentation history) febrile visits from the pre- and post- vaccination periods were divided into equal sized training and validation sets. We then fitted stepwise logistic regression model for the training set, considering as candidate independent variables age, gender, season of occurrence, clinical features (signs and symptoms) and Widal results, and taking the occurrence or non-occurrence of blood culture positive for *S.* typhi as the dependent variable. The final logistic regression equation included the following variables: age, abdominal pain, body temperature, presence of headache, and Widal serology. The coefficients for these final independent variables together with the intercept allowed calculation of the probability that each subject had culture-positive typhoid fever.

We examined different candidate probabilities that would serve as cut-offs for defining clinical typhoid in both the training and validation visits. We took as a cut-off probability, the value at or above which the number of predicted typhoid cases that were not culture positive was approximately equal to the number of blood culture confirmed typhoid cases. This strategy was in line with the previous studies demonstrating that blood cultures have ca. 50% sensitivity in detecting typhoid fever. The optimal cut-off value (≥0.12) was similar for training and validation datasets. We then examined whether this value was equally applicable to treatment visits occurring before and after vaccination. Because the appropriate cut-off value was higher for post-vaccination visits than for pre-vaccination visits, we took the more conservative post-vaccination cut-off (≥.13) as the final probability for defining a clinical typhoid visit for treatment of persistent fever. As noted earlier, any febrile episode in which there was no culture positive visit for *S.* typhi or *S.* paratyphi and in which there was at least one visit meeting the definition of clinical typhoid was taken as a clinical typhoid episode.

6. Management of events with uncertain data

There may be uncertainties in the date of exit (migration out of the study area, death) or the date of entry (births, in-migrations) of some individuals because the demographic information relies on recalling the events at the time of census, often by current residents or neighbors who may not have accurate information of the events. We have cross checked the dates for these events noted in the censuses using alternative data sources (for deaths: verbal autopsy forms, community health worker (CHW) logbooks, and clinic visits; for out-migrations: clinic visits and vaccination files; for births and in-migrations: clinic visits).

In the event of inconsistencies of the dates of these events from these different data sources, it was necessary to resolve the inconsistencies according to rules that established a hierarchy of reliability of information according to the data source. We used the following hierarchy of reliability of dates (from most to least reliable): 1. Verbal autopsies; 2. CHW logbooks; 3. Clinic visits; 4. Vaccination files; 5. Census. The algorithms used for implementation of these rules are given in the appendix.

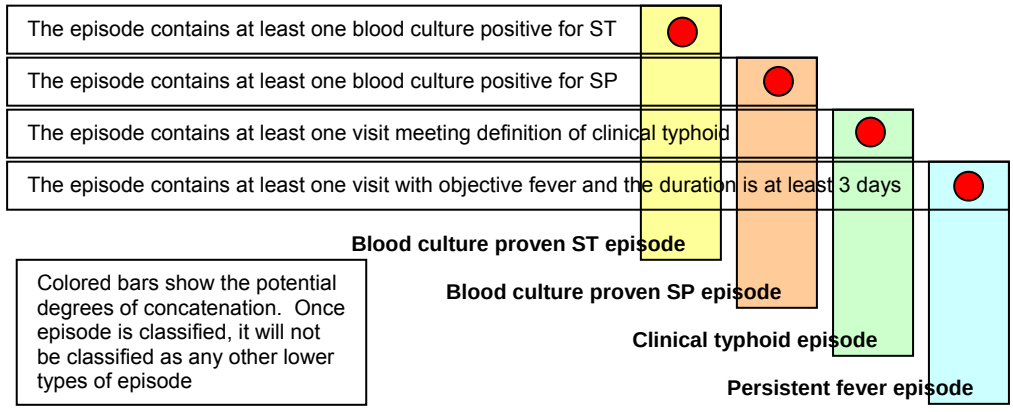
If the dating of exit events using these rules placed the date of exit before *zero time*, the subject was not considered to be present at *zero time* regardless of the listing of the subject in the *zero time* vaccination file.

7. Concatenation of febrile visits and hierarchical classification of concatenated episodes

Visits for subjective or objective fever were concatenated into single episode if the recalled date of onset of fever for one visit was within 14 days of the date of presentation from the previous visit. For purposes of the analysis, persistent febrile episodes were those in which at least one component visit had objective fever and the duration between the onset of first component visit and the date of presentation for the last component visit was at least 3 days.

To define the different principle outcome events in the analysis of vaccine protection, it was necessary to employ a hierarchical classification scheme, shown below. Once an episode was assigned to a particular outcome at one level of the hierarchy, the episode could not also be assigned to any level below it. Please see the definition section for explanation of terms

FIGURE – DESCRIPTIONS OF HIERARCHICAL CLASSIFICATION



8. Relationship between zero time, inclusion of events, and calculation of person-time of follow-up

The date of follow-up for all subjects was *zero time* (see definition section). However, depending on the type of outcome event, inclusion of outcome event in the analyses depended on the relationship between the date of *zero time* and the date of onset of the event. For analyses of vaccine protection against illnesses (blood culture proven *S.* typhi episodes, blood culture proven *S.* paratyphi episodes, clinical typhoid episodes, and persistent fever episodes), we included only episodes whose onsets occurred after *zero time*, to exclude those illnesses that were present at the time of vaccination. For

all other events noted below, we included all events whose onsets began from *zero time*. Follow-up time was also calculated from *zero time*.

	Vaccinee	**Non-Vaccinee**
Zero time	The date of vaccination	The median date of vaccination of the person's assigned cluster
Onset of illness	≥ *Zero time* + 1 day	≥ *Zero time* + 1 day
Date of migration out	≥ *Zero time*	≥ *Zero time*
Date of death	≥ *Zero time*	≥ *Zero time*
Beginning of follow-up	*Zero time*	*Zero time*

9. Principal analyses

Analyses of protection by Vi polysaccharide vaccine: These analyses considered total, indirect, and overall protection. Outcomes for these analyses included both those for which protection by Vi was anticipated (*S.* typhi episodes, clinical typhoid episodes, and episodes of persistent fever) as well as against *S.* paratyphi episodes, for which protection by Vi was not anticipated. The latter outcome was analyzed to evaluate the possibility of bias in the trial (reflected by protection against *S.* paratyphi episodes by Vi polysaccharide), as well as to evaluate whether immunization by Vi is associated with a replacement of typhoid fever by paratyphoid fever (reflected by an elevated risk of *S.* paratyphi episodes associated with Vi polysaccharide).

Total protection
Total protection is estimated by comparing the incidence of the outcome among Vi recipients and the incidence of the outcome among Hepatitis A vaccine recipients. It reflects the combination of direct protection of vaccinees by Vi together with herd protection of vaccinees in Vi-vaccinated clusters. For each type of event considered in primary analyses, we focused only on the initial event experienced by an individual.

Numerator episodes for analysis:

1. Culture positive typhoid episodes
Culture positive typhoid episodes are defined above. To be included in the analysis, identities of patients with such episodes must have been confirmed by home visits, conducted 7 days after presentation and the onsets of such episodes must have been between *zero time* plus one day and *zero time* plus 730 days (unless earlier censored as described below for the denominator).

2. Culture positive paratyphoid episodes
Culture positive paratyphoid episodes are defined above. To be included in the analysis, identities of patients with such episodes must have been confirmed by home visits, conducted 7 days after presentation and the onsets of such episodes must have been between *zero time* plus one day and *zero time* plus 730 days (unless earlier censored as described below for the denominator).

3. Clinical Typhoid episodes
Clinical typhoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day and *zero time* plus 730 days (unless earlier censored as described below for the denominator).

4. Persistent fever episodes
Persistent fever episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day and *zero time* plus 730 days (unless earlier censored as described below for the denominator).

Denominator for analysis:

Subjects in the denominator for analysis are those who were age- and otherwise eligible to receive a vaccine and were noted as having received either Vi or Hepatitis A vaccine.

Subjects noted as having been vaccinated but whose date of exit was ascertained to be before vaccination (*vida supra*, see appendix) are excluded. In analyses of time-to-event, the person-time at risk begins at *zero time* and continues until the date of death, date of out-migration, date of internal migration, date of event under analysis, or *zero time* plus 730 days, which ever came first.

Indirect protection

Indirect protection is estimated by comparing the incidence of the outcome among non-recipients of Vi in the Vi clusters and the incidence of the outcome among non-recipients of Hepatitis A vaccine in the Hepatitis A vaccine clusters. It reflects the herd protection of non-vaccinees in Vi-vaccinated clusters. For each type of event considered in primary analyses, we focused only on the initial event experienced by an individual.

Numerator episodes for analysis:

1. Culture positive typhoid episodes

Culture positive typhoid episodes are defined above. To be included in the analysis, identities of patients with such episodes must have been confirmed by home visits, conducted 7 days after presentation. The onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator).

2. Culture positive paratyphoid episodes

Culture positive paratyphoid episodes are defined above. To be included in the analysis, identities of patients with such episodes must have been confirmed by home visits, conducted 7 days after presentation. The onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator).

3. Clinical typhoid episodes

Clinical typhoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator).

4. Persistent fever episodes

Persistent fever episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator).

Denominator for analysis:

Subjects in the denominator for analysis will be all those who were noted as having received neither Vi nor Hepatitis A vaccine but were residents of the study clusters at *zero time*. In analyses of time-to-event, the person-time at risk begins at *zero time*, and continues until the date of death, date of out-migration, date of internal migration, date of event under analysis, or *zero time* plus 730 days, which ever comes first.

Overall protection

Overall protection is estimated by comparing the incidence of the outcome among population in the Vi clusters and the incidence of the outcome among population in the Hepatitis A vaccine clusters. It reflects the protection of the general population regardless of vaccine uptake.

Numerator episodes for analysis:

1. Culture positive typhoid episodes

Culture positive typhoid episodes are defined above. To be included in the analysis, identities of patients with such episodes must have been confirmed by home visits, conducted 7 days after presentation. The onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator).

2. Culture positive paratyphoid episodes
Culture positive paratyphoid episodes are defined above. To be included in the analysis, identities of patients with such episodes must have been confirmed by home visits, conducted 7 days after presentation. The onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator).

3. Clinical Typhoid episodes
Clinical typhoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator).

4. Persistent fever episodes
Persistent fever episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator).

Denominator for analysis:

Subjects in the denominator for analysis will be all those present at *zero time.* The person-time at risk begins at *zero time* and continues until the date of death, date of out-migration, date of internal migration, date of event under analysis, or *zero time* plus 730 days, which ever comes first.

Analysis of safety
Surveillance for safety consisted of the following:

I. Observation 15-30 minutes after vaccination
All vaccine recipients were asked to remain at the vaccination site for 30 minutes immediately after vaccination, and all local and systemic adverse events were noted.

II. Adverse events 3 days after vaccination
A sub-sample of 320 participants was visited at home daily for 3 days after vaccination. At each visit, all adverse events observed by the trained study personnel or reported by the parents/guardians of the subject were recorded.

III. Other adverse events in the month after vaccination
Vaccinees were asked to attend designated project health facilities including all hospitals serving the study population if local or systemic events occurred during the month after immunization. A special study form was completed for every case observed, and a senior study physician made an assessment of causal relationship between receipt of the study vaccine and the occurrence of the adverse event.

IV. Surveillance of deaths in the first month following vaccination
All deaths among trial participants during the follow-up were identified through the health facilities and censuses carried out after vaccination. A special study verbal autopsy form was completed for every death.

Separate analyses are conducted for each of these four categories of events. In each of these analyses, the risk of the cited event will be compared for recipients of Vi vaccine versus recipients of Hepatitis A vaccine. Events are categorized as severe or non-severe using standard ICH-GCP definitions. All events are analyzed, regardless of the physicians' judgments about causality. For all categories of events, events whose onset occurred on or after *zero time* will be considered. For categories I, III and IV, the denominator will be the entire vaccinated population at risk. For category II, the denominator will be the sub-sample of vaccinees who were followed up at home.

Analysis of immune responses

A sub-sample of vaccinees was bled immediately before vaccination, 42 days after *zero time*, and again at two years after *zero time*. Sera from these samples were assessed for IgG anti-Vi antibody titers and antibodies against Hepatitis A (anti-HAV) at GSK laboratories in Rixensart, Belgium, blinded to the identity of the vaccine that each subject had received.

Analyses compare recipients of Vi and Hepatitis A vaccine for geometric mean titers (GMT) and the proportion with protective titers at each bleed, and for the proportions seroconverting between baseline and bleed 2, and between baseline and bleed 3.

10. Statistical analyses

The primary analysis for the study was of total protection by Vi against culture confirmed *S.* typhi episodes amongst all vaccinees during the entire period of follow up. For subjects having more than one such episode, only protection against the first episode is considered. Subgroup analyses of total protection, analyses of total protection by Vi against other outcomes (culture confirmed *S.* paratyphi, clinical typhoid fever, and persistent fever), as well as analyses of indirect and overall protection against each of the four outcomes were considered secondary analyses.

Crude bivariate comparisons
Crude bivariate, two-group comparisons employed Pearson's Chi squared test for categorical outcomes and Student's t-test for dimensional outcomes. Crude bivariate, two-group comparisons of survival data compared time-to-event with use of Kaplan-Meier curve, assessed statistically with the log rank test.

Bivariate comparisons adjusted for cluster-effects
To adjust the crude bivariate, two-group comparisons of dichotomous outcomes for the cluster-based design of the trial, we employed GEE (Generalized Estimation Equations) with the logit link function. To adjust the two-group comparison of survival data for clustering, we fitted marginal Cox proportional hazard models that adjusted for cluster-effects, after first ascertaining that the proportional hazard assumption was fulfilled, with the use of Kolmogorov test.

Two-group comparisons jointly adjusted for cluster effects and covariates
Joint adjustment of two-group comparisons for cluster effects and baseline covariates employed the models cited in previous paragraph with inclusion of covariates of interest as independent variables. The primary analysis of the trial cited above refers to the analysis of total protection jointly adjusted for cluster effects and selected covariates (see next paragraph for selection criteria).

Selection of baseline covariates for multivariable models
Variables ascertained at baseline (*zero time* for subjects who were present at *zero time*) were included in the multivariable models if, in bivariate, cluster adjusted analyses, the variable was associated ($P \leq 0.10$; two-tailed) with the occurrence of the outcome in the entire study population under analysis.

P value thresholds
A P value less than 0.05 (two-tailed) was considered statistically significant for all analyses.

11. Analysis specifics

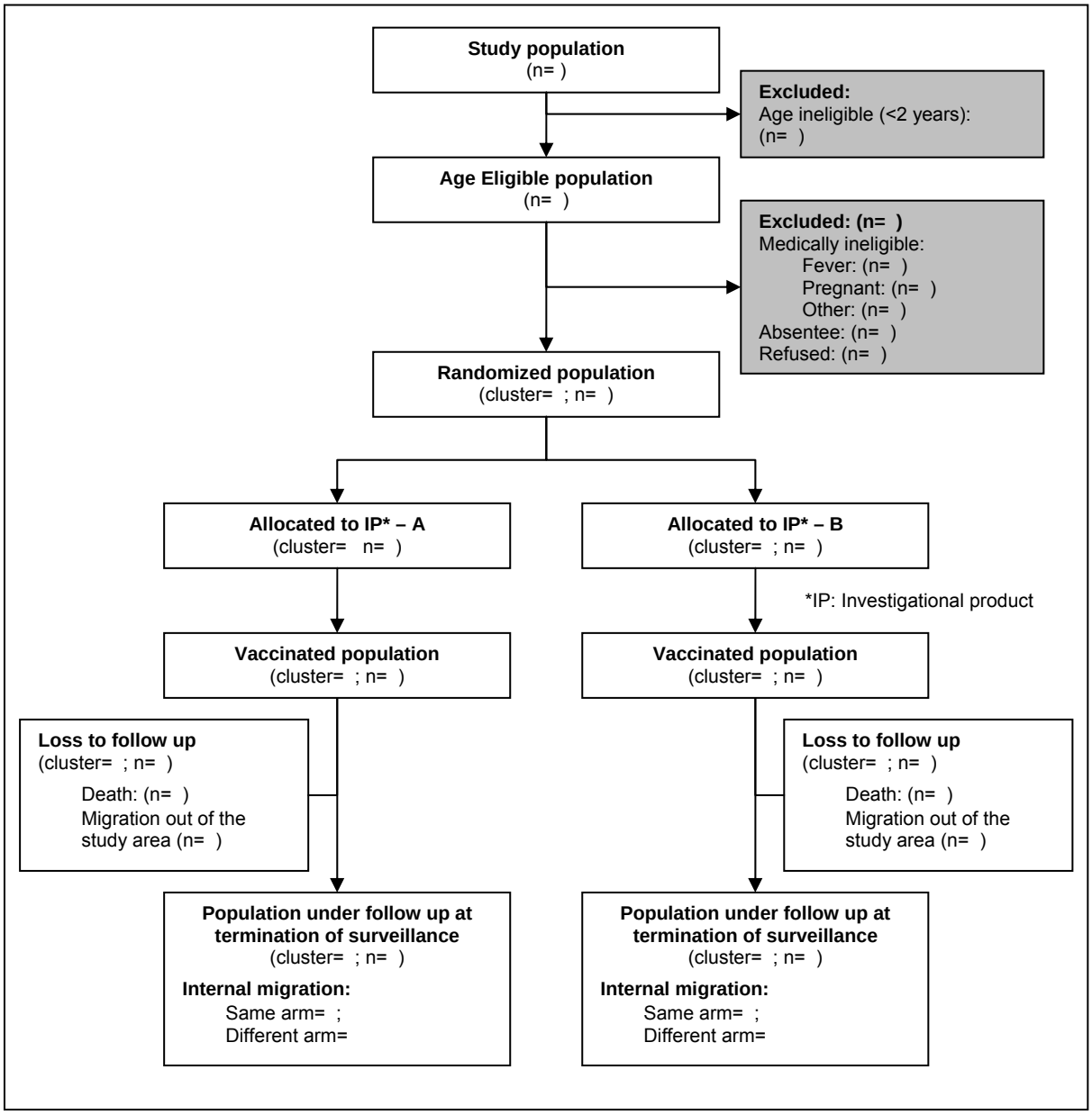
Prior to unblinding of the codes for the trial, all analyses will have been conducted on a "re-randomized data set", in which the 80 clusters for the trial have been randomly reassigned to 2 groups, each with 40 clusters. This was done to facilitate development of an analysis plan in a fashion that was not influenced by knowledge of which vaccine had been received, and also that allowed creation of the complete ensemble of analytic programs prior to unblinding.

Assembly of persons and events
Contemporary medical journals require that papers conform to the guidelines in the Consolidated Standards of Reporting Trials (CONSORT) statement. The statement includes a checklist of items that should be included in the trial report. In addition, the CONSORT statement recommends including a flow diagram to show the flow of participants from group assignment through to the final analysis.

The special flow diagram proposed for denominators for cluster-randomized trials has been proposed, and we have adapted the diagram for this trial. In addition, we have developed a corresponding flow diagram for numerator events under analysis.

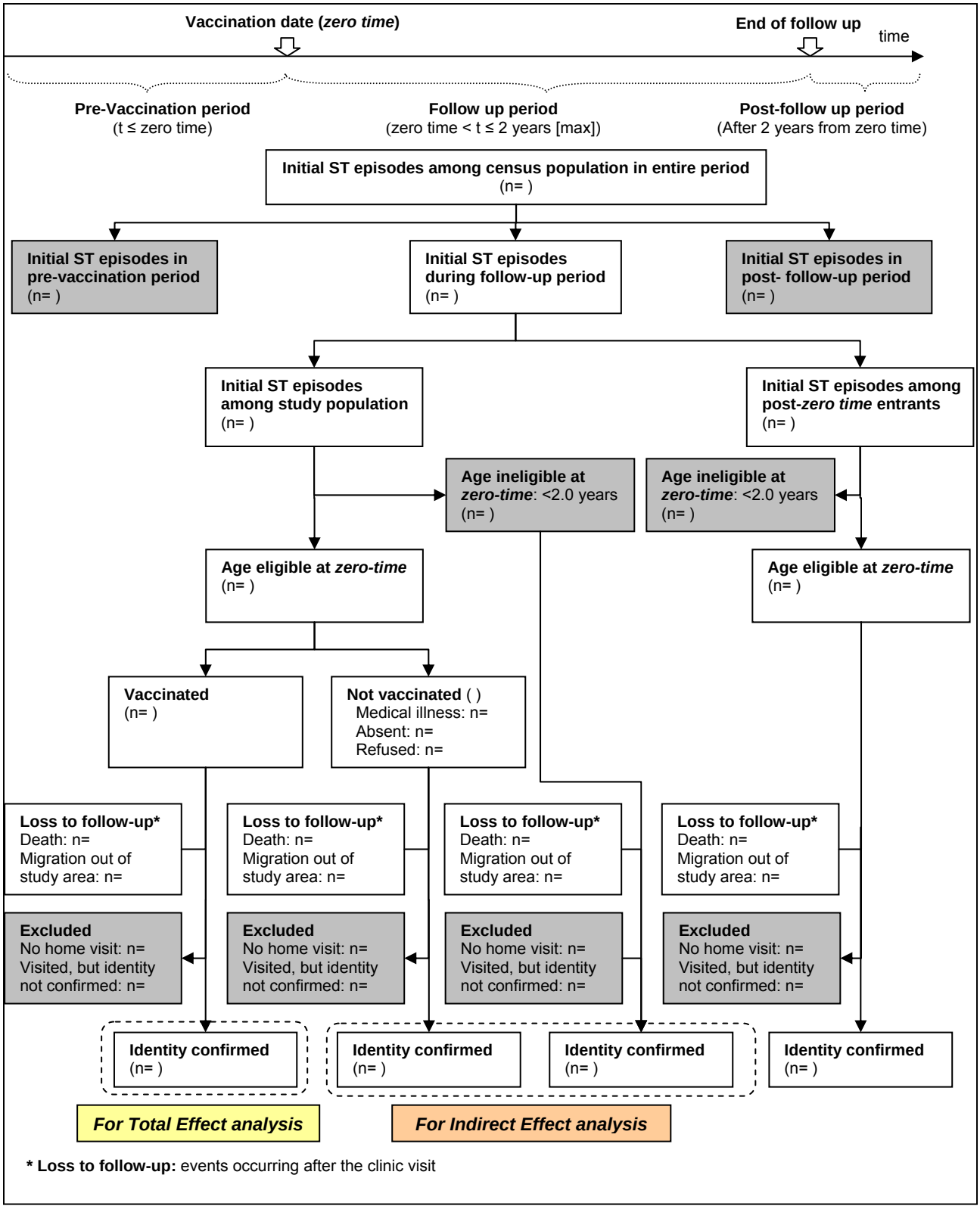
1. CONSORT chart for denominators

This chart represents the flow of study population from selection and randomization to final follow up showing the loss to follow up.



2. Case flow charts for numerators

In contrast to the CONSORT chart for the denominators, the case flow chart shows the flow of cases (numerators) detected during the whole study period. This is prepared for all outcomes of interests: culture-confirmed *S.* typhi and *S.* paratyphi, clinical typhoid, and persistent fever. Below we show the diagram for the *S.* typhi (ST) episodes that were included in the analyses.



Candidate zero time variables for inclusion as covariates in final multivariable, Cox regression models of Vi vaccine protection

Candidate *zero time* variables for inclusion as covariates in final multivariable, Cox regression models of Vi vaccine protection are listed below. Note that several of variables measure the same construct (e.g. socio-economic status, status of drinking water and hygiene). In instances in which several

variables measuring the same construct meet the criteria for inclusion in the final model (*vide supra*) we selected only the variable most strongly associated with the outcome for the model.

Candidate variables and their categories:

Variable	**Categories**
Age	In years (continuous)
Gender	0: female 1: male
Hindus	0: others 1: hindu)
Individuals living in a household with literate household head	0: No 1: Yes
Individuals living in a household with household head having more than 5 years of schooling	0: No 1: Yes
Individuals living in a household with important economic contributor having stable occupation	0: others 1: Stable: professional, service, shop owner, or farm owner
Individuals living in a household using safe toilet	0: others 1: Safe toilet: use of own (non-shared) flush toilet
Individuals living in a household using safe water source (tap at household level)	0: No 1: Yes
Individuals living in a household using safe water source (tap or hand pump at household level)	0: No 1: Yes
Individuals living in a household using boiled or filtered water	0: No 1: Yes
Individuals living in a household always wash hands with soap and water after defecation	0: No 1: Yes
Individuals living in a household having specific place for waste disposal	0: No 1: Yes
Individuals living in owned house	0: No 1: Yes
Individuals living in a household owning refrigerator	0: No 1: Yes
Individuals living in a household owning motorbike	0: No 1: Yes
Individuals living in a household owning telephone	0: No 1: Yes
Individuals living in a household owning all luxury items	0: No 1: Yes; items: Refrigerator, motorbike, and washing machine
Individuals living in a household owning at least one luxury item	0: No 1: Yes; items: Refrigerator, motorbike, and/or washing machine
Monthly household income	In Indian Rupee (continuous)
Individuals with high monthly household income	0: On/Below median household monthly income 1: Above median household monthly income
Per-capita monthly income	In Indian Rupee (continuous)
Individuals with high per-capita monthly income	0: On/Below median household per-capita monthly income 1: Above median household per-capita monthly income
Cluster size	No. of age eligible individuals (continuous)
Individuals living in larger cluster	0: On/Below median cluster size 1: Above median cluster size
Household size	No. of total individual (continuous)
Population density /10m^2	Total study population/area (continuous)
Higher population density /10m^2	0: On/Below median population density 1: Above median population density

Distance (m) from the household to the nearest health outpost	Distance in meters (continuous)
Longer distance from the household to the health outpost	0: On/Below median distance to the outpost 1: Above median distance to the outpost
Larger cluster size of adults at stratification	0: <500 adults (age >18 years) per cluster at stratification 1: ≥500 adults per cluster at stratification
Larger cluster size of pediatrics at stratification	0: <200 children (age ≤18 years) per cluster at stratification 1: ≥200 children per cluster at stratification
Pre-vaccination incidence of typhoid fever	0: On/Below median pre-vaccination typhoid incidence 1: Above median pre-vaccination typhoid incidence

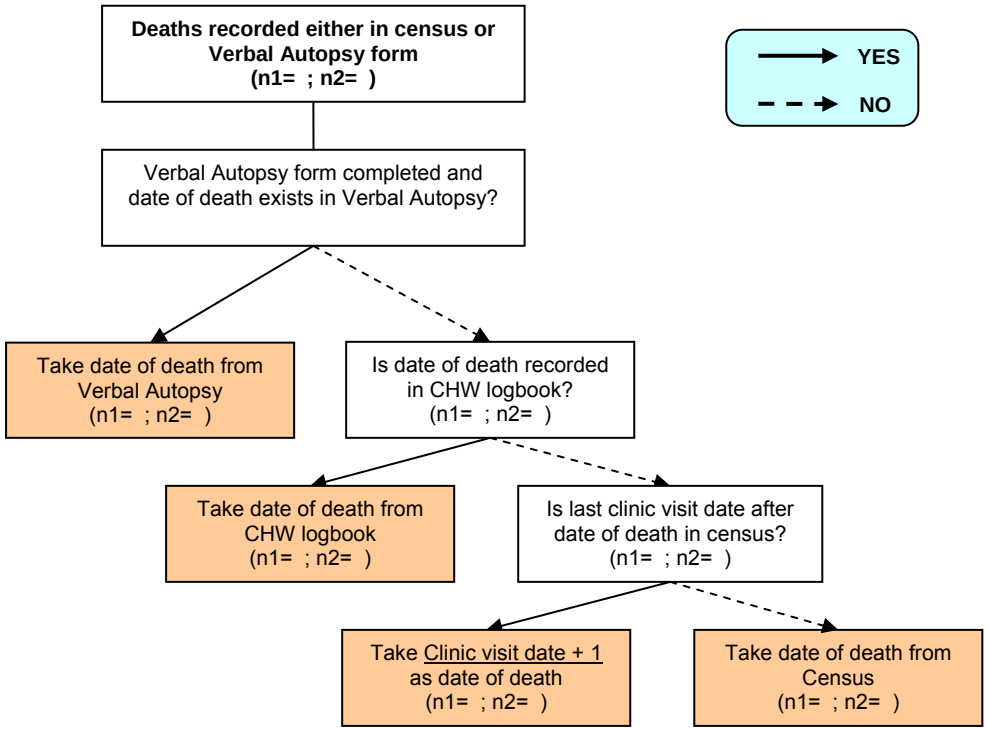
Subgroup analyses:
We prespecified several zero time and post-zero time variables and their categories for conduct of subgroup analyses of vaccine protection. The pre-specified variables and their categories are given below.

Variables and their categories used for subgroup analyses:
These variables were selected based on aforementioned criteria using the re-randomized data

Variable	**Categories**
Age at zero time	1. 2-4.9 years 2. 5-14.9 years 3. 15 years +
Gender	0. Female 1. Male
Socio-economic status (SES)	0. Below median per-capita household monthly income 1. On/Above median per-capita household monthly income
Safe water source	0. Households without own tap/hand pump 1. Households with own tap/hand pump
Religion	0. Others 1. Hindu
Population density	0. Below median cluster population density 1. On/Above median cluster population density
Pre-vaccination incidence of typhoid fever for the cluster	0. Below median pre-vaccination typhoid incidence 1. On/above median pre-vaccination typhoid incidence
Vaccination coverage	1. First tertile of coverage of the entire cluster by either vaccine 2. Second tertile of coverage of the entire cluster by either vaccine 3. Third tertile of coverage of the entire cluster by either vaccine
Year of follow-up	0. Onset between zero time (ZT) and ZT+365 1. Onset between ZT+366 and ZT+730

APPENDIX: Demographic date assignment flowchart for KOLKATA TYPHOID TRIAL

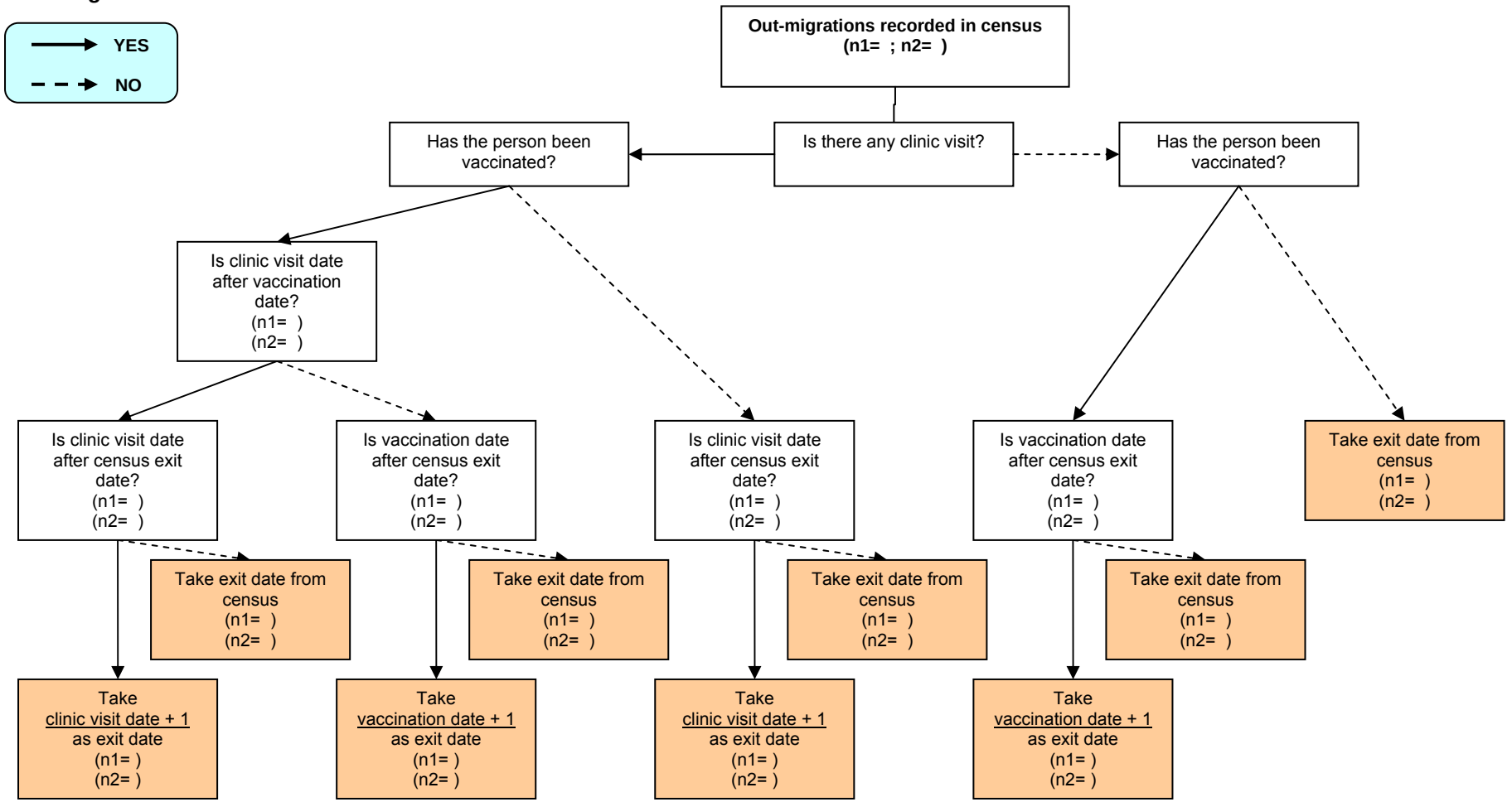
1. Deaths



Footnote:

- n1 is the number from whole period
- n2 is the number from at or after zero time
- We do not consider vaccination status in adjusting the date of death, since it would arbitrary place the death to the day after receiving the vaccine
- Reclassification of date of death can only move the date forward in time or stay the same

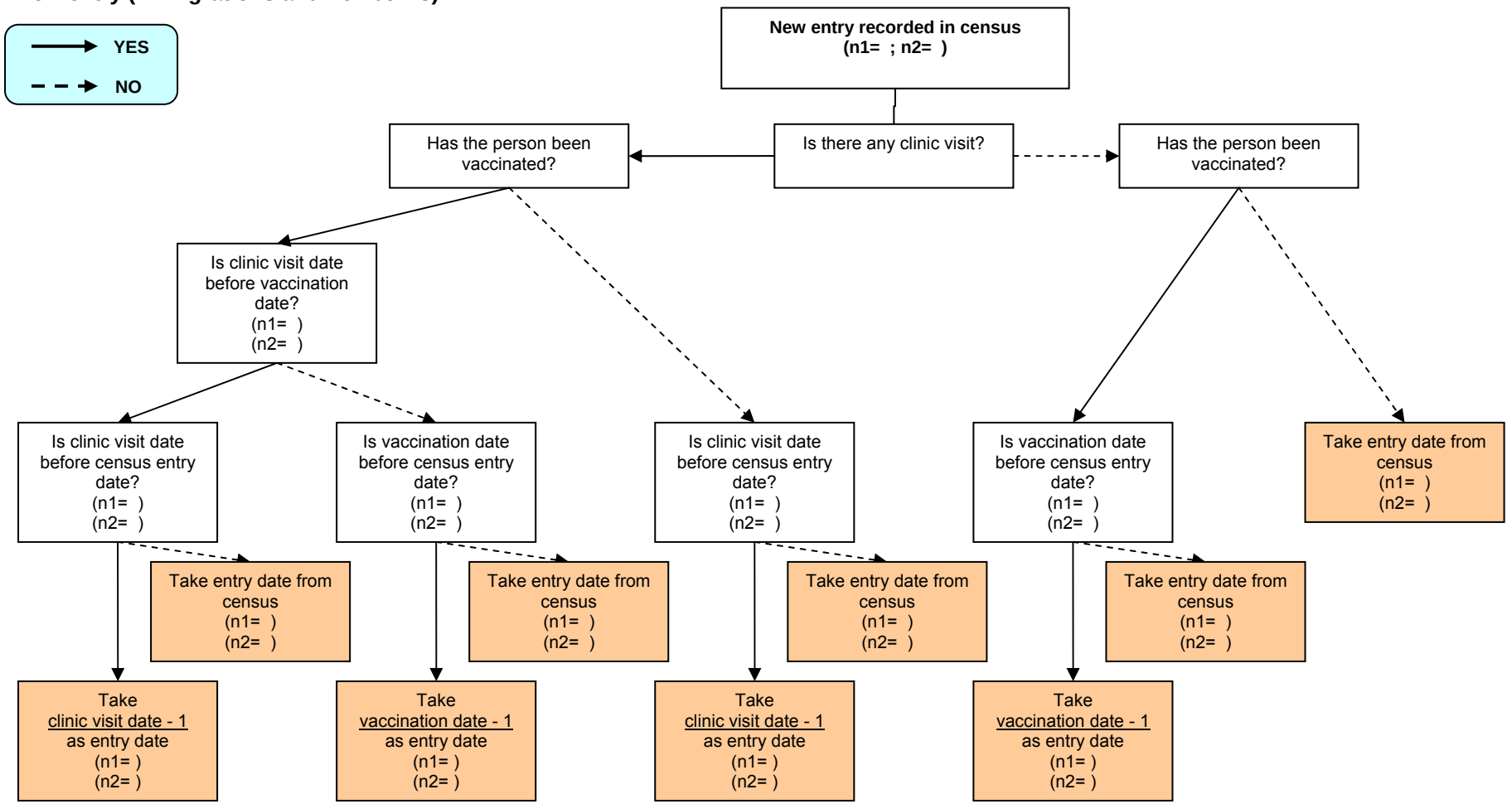
2. Out-migrations



Footnote:

- n1 is the number from whole period
- n2 is the number from at or after zero time
- Not all CHW logbooks recorded the out-migration events, thus we did not use CHW logbook to assess/define the date of out-migrations
- Reclassification of date of migration out can only move the date forward in time or stay the same

3. New entry (In-migrations and newborns)



Footnote:

- New entry includes both in-migration and being born
- n1 is the number from whole period
- n2 is the number from at or after zero time
- CHW logbooks did not record the entry events systematically, thus we did not use CHW logbook to assess/define the date of entry
- Reclassification of date of entry can only move the date backward in time or stay the same

International Vaccine Institute
Diseases of the Most Impoverished (DOMI)
Typhoid Program

Analytical Plan of the Vi Effectiveness Trials Karachi, Pakistan

September/2008

1. Overview

1.1 Corresponding protocol and its identification:

Efficacy of a combined Vi vaccination and health education program on reducing the burden of typhoid during childhood: a demonstration project in Karachi (Pakistan) (NCT00125047)

The study has been registered with the US clinical trial registry (http://www.clinicaltrials.gov) which is mandated for publication of clinical trial results. Registration number is given in the parentheses.

1.2 Investigational product:

Vi capsular polysaccharide vaccine

1.3 Indication:

Prevention of infection by *Salmonella* Typhi

1.4 Sponsor:

The Disease of the Most Impoverished Program – Funded by the Bill and Melinda Gates Foundation and administered by the International Vaccine Institute

The DOMI Program
International Vaccine Institute
San 4-8, Bongcheon-7 dong, Kwanak-gu
Seoul, Korea 151-919
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Fax. +82-2-872-2803

1.5 Study phase:

Phase 3b Effectiveness evaluation (multi-site)

1.6 Principal Investigators:

Zulfiqar A Bhutta – Department of Pediatrics, Aga Khan University
John D Clemens – International Vaccine Institute

1.7 Co-Investigators:

Qamaruddin Nizami – Department of Pediatrics, Aga Khan University
R Leon Ochiai – International Vaccine Institute
Mohammad Ali – International Vaccine Institute

1.8 Summary:

This document describes the specific procedures to be followed in the statistical analysis of the above-mentioned trial and the format of data output. The primary features of the statistical analysis plan were specified in the original protocol. This document focuses on the statistical issues related to the primary analysis of vaccine protection (effectiveness).

2. Study Synopsis

TITLE	**Efficacy of a combined Vi vaccination and health education program on reducing the burden of typhoid during childhood: a demonstration project in Karachi (Pakistan)**
STUDY OBJECTIVES	**Primary** 1. To determine total protection conferred by Vi polysaccharide vaccine, given in a 1-dose schedule to children aged 2 to 16, against episodes of blood-culture confirmed *S. typhi* **Secondary:** 2. To determine total protection conferred by Vi against episodes of blood-culture confirmed *S. paratyphi* 3. To determine total protection conferred by Vi against episodes of persistent fever 4. To determine indirect protection conferred by Vi against episodes of blood culture-confirmed *S. typhi, S. paratyphi*, and persistent fever 5. To determine overall protection conferred by Vi against episodes of blood culture-confirmed *S. typhi, S. paratyphi*, and persistent fever 6. To evaluate the safety and immunogenicity of Vi vaccine following administration through a mass vaccination campaign. 7. To estimate the logistic feasibility of a mass immunization campaign, 8. To evaluate knowledge, attitudes, beliefs and practices among the residents and health providers in the study area regarding Vi vaccination and typhoid, and 9. To estimate the cost-effectiveness of preventing typhoid fever with Vi vaccine
TRIAL LOCATION	Hijrat Colony, Sultanabad, and Bilal Colony, Karachi, Pakistan
STUDY PERIOD	**Hijrat and Sultanabad:** Dec 2001-Jul 2003 Pilot Phase (18 months) approved previously by WHO and local ethical committee Aug 2003-Sep 2005 Vaccination Phase (26 months) **Bilal:** Mar 2003-Jul 2004 Pilot Phase (15 months) approved previously by WHO and local ethical committee Nov 2004-Dec 2006 Vaccination Phase (26 months)
STUDY DESIGN	Evaluation-blinded cluster-randomized control effectiveness trial
STUDY SUBJECTS	Inclusion criteria • Permanent residents, registered in the project censuses • Provide informed consent to receive vaccines • Age: ≥ 2 years and ≤16 years Exclusion criteria • Fever (>37.5° C, axillary) • Pregnancy • Lactating
SAMPLE SIZE ESTIMATION	A total of approximately 24,834 participants will be needed in order to have 80% power to detect a 60% vaccine protection at a 5% level of significance (two-tailed). This sample size calculation assumed a minimum cumulative typhoid incidence of 2.8 per 1,000 (during 2 years) and between cluster coefficient of variation (CV) of 0.5. Assuming an annual attrition rate of 10% a total sample of 27,592 participants is needed. Each cluster will be composed of groups of households. Cluster size will be 200-600 individuals (120 clusters).

STUDY VACCINES	***Investigational product:*** **Vi polysaccharide typhoid vaccine**, Typherix ® (GlaxoSmithKline) **Form**: Clear liquid in a 1 dose pre-filled syringe **Dose**: single 0.5ml dose (25ug of the purified Vi polysaccharide of *S.* typhi) for both children and adults **Route**: IM in the deltoid area ***Active Control:*** **Hepatitis A vaccine:** Havrix ® (GlaxoSmithKline) **Form**: Suspension liquid in a 1 dose pre-filled syringe. **Dose**: single 0.5ml dose (720 EL.U of viral antigen) **Route**: IM in the deltoid area Both study vaccine and active control vaccine had same appearances and were supplied in identical form (0.5ml single-dose pre-filled syringe).
CODES	C, M
CO-INTERVENTION	All subjects were instructed about signs and symptoms of typhoid and about seeking appropriate healthcare in the event these signs and symptoms are present.
EVALUATION CRITERIA	<u>Effectiveness</u> Total, indirect, and overall protection by Vi polysaccharide vaccine against: • Episodes of fever in which *S.* typhi is isolated from blood culture. • Episodes of persistent fever <u>Safety</u> • Local and systemic events detected in first 3 days after immunization via active surveillance (subset of 480 subjects) • Clinical events detected by passive surveillance in the first month after vaccination (entire study population) • Deaths occurring in the first month following vaccination (entire study population) <u>Vi Immunogenicity (N: 480, same subset as above)</u> • Levels of serum IgG anti-Vi antibody titers (GMT) • Seroconversion rate (defined as greater than or equal to 4-fold rise of serum antibodies in bleed 2 relative to bleed 1) • Seroprotection rate (defined as attainment of a titer of serum antibodies of greater than or equal to 1µg/ml) <u>Vaccine logistics, costs and feasibility</u> • Resources, including personnel, needed for vaccine procurement, storage, transport and vaccine delivery, • Efficiency of vaccine storage, transport, and handling • Safe vaccination practices including vaccine administration and disposal of sharps • Detailed costing of vaccine delivery (project and vaccination level) • Vaccination costs (public and private)
DURATION OF STUDY PERIOD (per subject)	2 years after vaccination

3. Description of the Study sites

The project took place in three squatter settlements in Karachi, Pakistan. Two large adjacent squatter settlements called Sultanabad and Hijrat Colony were included in the study in 2001. The other settlement, the Bilal colony, which is in the industrial area, was included in the study in 2003. Bilal Colony site was included later during the project, since the incidence rate in the first two sites may not have been enough to meet the statistical power required.

4. *A priori* definitions

Cluster: The study was a cluster randomized trial. The unit of the randomization was a *cluster*. A cluster was a collection of geographically contiguous households with defined boundaries given by the project using a geographic information system (GIS) database. There were 120 clusters of similar population size: 60 clusters in Hijrat and Sultanabad, and 60 in Bilal.

Zero time: The *zero time* is the beginning of the study period defined separately for vaccinees and non-vaccinees. For vaccinees, the *zero time* is the date of vaccination; and for non-vaccinees, it is the median date of vaccination of the persons vaccinated in the cluster.

Age- and residence- eligibility for the trial: Persons present at the time of vaccination, as reflected in the pre-vaccination census, and aged between 2.0 and 16.0 years at the *zero time* were eligible to receive the vaccine.

Medical exclusion criteria for the trial: Individuals with one or more medical contraindications to either of the vaccines at *zero* time were excluded. These contraindications included acute fever, lactation and pregnancy.

Absentees: Absentees were people known to reside in the field area during vaccination but failed to be contacted by teams recruiting subjects for vaccination. The vaccination period was approximately 5 weeks long. These subjects were either in the pre-vaccination census or were noted retrospectively in the close-out census to have been residing in the study area at the time of vaccination.

Refusers: The trial required signed informed consent for participation. Children whose parents refused to sign informed consent for participation were considered refusers.

Censuses: Three censuses were carried out to collect the household information at different time points. These censuses captured the *de jure* population (defined as persons who stated that their residence in the study area was their customary residence) and recorded the demographic events of the population in the study area. Two censuses were conducted prior to the vaccination, one (initial census) at the onset of pre-vaccination surveillance period and the other (pre-vaccination census) just before vaccination. Third census was conducted after the two-year follow-up period to update the study area population through vital demographic events. The final census was termed the close-out census.

Populations with specific demographic events for the analysis:

In-migrants: Individuals who were not residents at *zero time* but were identified in the close-out census as having moved into the study area were termed in-migrants.

Newborns: Individuals who were not residents at *zero time* but were identified in the close-out census as having been born in the study area were termed newborns.

Out-migrants: Individuals who were residents of the study area at *zero time* but were identified in the close-out census having moved out of the study area were termed out-migrants.

Internal-migrants: Individuals who were residents of the study area at *zero time* but were identified in the close-out census as having changed their residences in the study area were termed internal-migrants.

Randomized population: Individuals who were age-eligible and known to be present at *zero time*, either by being in the pre-vaccination census or by being retrospectively identified as having been present at *zero time* in the post-vaccination censuses, were considered randomized according to the assignment of the cluster of residence at *zero time*.

Age- and residence- eligible population for surveillance: Persons present in the pre-vaccination census, and aged between 2 and 18 years at the time of onset of the disease during the follow up period were eligible to be included in the analysis.

Vaccinee: A member of randomized population who received assigned agent.

Loss to follow up: Those who moved out from the study area during the follow up period.

Pre-vaccination period: Pre-vaccination period is the period before the *zero time*.

Study follow-up period: Follow-up for typhoid fever and paratyphoid fever commenced from the day after the *zero time*. Follow-up for out-migration and death began at *zero time*. The maximum duration of follow-up was 2 years (730 days) after *zero time*, which was considered the termination date of surveillance.

Post-follow-up period: The time after the completion of follow-up period is defined as post-follow-up period. Onset of events occurring in this period will not be considered for analyses.

Definitions on fever:

Objective Fever: An axillary temperature ≥37.5°C.

Subjective Fever: Historical recall of having fever

Persistent fever visit: A report by a patient of having at least 3 days of fever at the time of presentation to the treatment center

Febrile episode: Visits for subjective or objective fever were concatenated into single episode if the recalled date of onset of fever for one visit was within 14 days of the date of presentation from the previous visit. The date of onset is taken as the recalled onset of fever for the first component visit of the episode.

Persistent fever episode: Febrile episodes with at least one component visit with objective fever were considered persistent if the duration between the onset of first component visit and the date of presentation of the last component visit was at least 3 days. The date of onset is taken as the recalled onset of fever for the first component visit of the episode.

Definitions on typhoid fever:

Blood culture proven typhoid fever: A subject was considered to have blood culture proven typhoid fever if at least one blood culture of the episode yielded a growth of *S.* typhi.

Typhoid fever episode: A febrile episode with blood culture proven typhoid fever was defined as typhoid fever episode. The date of onset is taken as the recalled onset of fever for the first component visit of the episode. We excluded episodes where identity check failed to confirm the identity of the person.

Definitions on paratyphoid fever:

Blood culture proven paratyphoid fever: A subject was considered to have blood culture proven paratyphoid fever if at least one blood culture of the episode yielded a growth of *S.* paratyphi A or B.

Paratyphoid fever episode: A febrile episode that is not typhoid fever episode, but has at least one blood culture positive for *S. paratyphi* A or B was defined as paratyphoid fever

episode. The date of onset is taken as the recalled onset of fever for the first component visit of the episode. We excluded episodes where identity check failed to confirm the identity of the person.

5. Randomization

Blocked randomization to study agents was done after stratifying the clusters based on information in the pre-vaccination census. This was done separately for Hijrat/Sultanabad and Bilal, since Bilal vaccination was done one year later. The strata considered the number of the targeted children in the cluster and site (Hijrat and Sultanabad). For Bilal, only the number of the targeted children in the cluster was considered. The strata were 1) Large cluster (n>350) in Sultanabad; 2) Small cluster (n≤350) in Sultanabad; 3) Large cluster (n>350) in Hijrat; 4) Small cluster (n≤350) in Hijrat; 5) Large cluster (n>450) in Bilal; 6) Small cluster (n≤450) in Bilal; where n was the median cluster size. Within each stratum, a table of random numbers was used by an external statistician who was blinded to the vaccine groups to allocate the 120 clusters to one of the two vaccines (60 clusters to each vaccine group).

6. Surveillance

The disease surveillance system had been set up at the beginning of the project. Age groups under surveillance were selected as those judged by local officials to be the most appropriate targets for typhoid vaccination: 2-16 year olds. However, during the 2-year post-vaccination follow up period, we had expanded the age group for surveillance to 2-18 years of age to accommodate the aging of vaccination target population (i.e. 2-16 year old at *zero time*).

Three project healthcare facilities were established (one in each site) and served as clinic and referral point for the patients. Passive surveillance was augmented through community health workers visiting each household weekly to encourage febrile patients to visit participating healthcare facilities, and private practitioners were encouraged to refer patients for free typhoid diagnosis and treatment at these centers. Additionally, private practitioners were encouraged to refer patients for free typhoid diagnosis and treatment at these project facilities, since the surveillance sites were considered insufficient to capture all cases from the communities. Patients aged 2-18 years, residing in the catchment areas, and presenting to a treatment facility with fever of 3 days or more were eligible to participate in the study. Surveillance included patients treated as outpatients as well as inpatients. Surveillance included both systematic collection of clinical information, recorded on standardized forms, and collection of a single blood culture by venipuncture for culture.

A crucial feature of the surveillance was accurate identification of all patients attending the treatment facilities to determine the study participants. To accomplish this, a computer-based identity search program was developed and equipped in 3 study treatment centers. The program allowed active identity search while the patient was being attended by the physician. This identification process was conducted by the project health personnel from the community. Cases from private sectors without active identity confirmation were checked with the same program at the data management unit retrospectively. Additionally, the culture positive cases were followed up to assess disability at days 7, 14, 30, and 60. At the time of the follow up on the day 7, study personnel were to confirm the identity of the individual so that no misclassification was to occur in the analyses.

7. Validation of blood culture isolates

Blood culture isolates of *S.* typhi and *S.* paratyphi identified by AKUH clinical laboratories during the study period have been checked for their identities at a reference lab. All *S.* typhi and *S.* paratyphi isolates were confirmed at the IVI lab and at the Korean National Institute of Health (Salmonella reference laboratory for Korea).

We defined that the initial assessments at the AKUH clinical laboratory were correct based on the observation of the lab's practice and the results of the retest done in presence of Anne-Laure and John Wain in July 2003 and Leon in February 2008. 4 discrepancies out of 75 retested samples at

reference lab conferred error rate of 5% (95% concordance), which is in the acceptable range for a clinical lab.

See Appendix for the details on specimen assessment conducted.

8. Management of events with uncertain data

There may be uncertainties in the date of exit (migration out of the study area, death) or the date of entry (births, in-migrations) of some individuals because the demographic information relies on recalling the events at the time of census, often by current residents or neighbors who may not have accurate information of the events. We have cross checked the dates for these events noted in the close-out census using alternative data sources (for deaths: verbal autopsy forms, vaccination files and clinic visits; for out-migrations: clinic visits and vaccination files; for births and in-migrations: clinic visits).

In the event of inconsistencies of the dates of these events from these different data sources, it was necessary to resolve the inconsistencies according to rules that established a hierarchy of reliability of information according to the data source. We used the following hierarchy of reliability of dates (from most to least reliable): 1. Verbal autopsies; 2. Clinic visits; 3. Vaccination files; 4. Census. The algorithms used for implementation of these rules are given in the appendix.

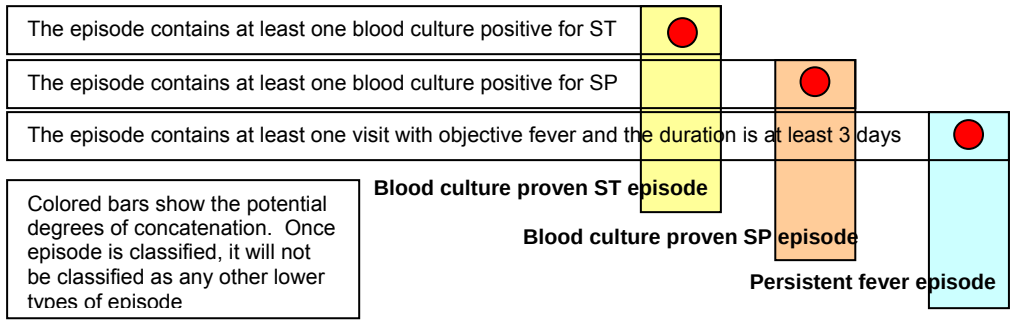
If the date of an exit event using these rules placed the date before *zero time*, the subject was not considered to be present at *zero time* regardless of the listing of the subject in the *zero time* vaccination file.

9. Concatenation of febrile visits and hierarchical classification of concatenated episodes

Visits for subjective or objective fever were concatenated into single episode if the recalled date of onset of fever of one visit was within 14 days of the date of presentation of the previous visit. For purposes of the analysis, persistent febrile episodes were those in which at least one component visit of the episode had objective fever and the duration between the onset of first component visit and the date of presentation of the last component visit was at least 3 days.

To define the different principle outcome events in the analysis of vaccine protection, it was necessary to employ a hierarchical classification scheme, shown below. When an episode was assigned to a particular outcome at one level of the hierarchy, the episode was not assigned to any level below it. Please see the definition section for explanation of terms

FIGURE – DESCRIPTIONS OF HIERARCHICAL CLASSIFICATION



10. Relationship between zero time, inclusion of events, and calculation of person-time of follow-up

The date of onset of follow-up for age- and residency- eligible subjects was *zero time* (see definition section). Those who were older and not eligible to receive the vaccine, but age- and residency-eligible for surveillance (i.e. age>16 and ≤18 years) also enter the study from *zero time*. Those who

were younger and not eligible to receive the vaccine enter the study when they reach 2 years of age during the follow up period (post *zero time* entrants). For post *zero time* entrants, we define this as date of entry to the study.

Age- and residency- eligible population for the trial at *ZT*
Depending on the type, inclusion of an outcome event in the analyses was dependent on the relationship between the date of *zero time* and the date of onset of the event. For analyses of vaccine protection against illnesses (blood culture proven *S.* typhi episodes, blood culture proven *S.* paratyphi episodes, and persistent fever episodes), we included only episodes whose onsets occurred after *zero time* and excluded those illnesses that were present at the time of vaccination. For all other events noted below, we included all events whose onsets began from *zero time*. Follow-up time was also calculated from *zero time*.

	Vaccinee	**Non-Vaccinee**
Zero time	The date of vaccination	The median date of vaccination of the person's assigned cluster
Onset of illness	≥ *Zero time* + 1 day	≥ *Zero time* + 1 day
Date of migration out	≥ *Zero time*	≥ *Zero time*
Date of death	≥ *Zero time*	≥ *Zero time*
Beginning of follow-up	*Zero time*	*Zero time*

Age ineligible population for vaccination, but age- and residency- eligible population for the surveillance
Depending on the type, inclusion of an outcome event in the analyses was dependent on the relationship between the date of entry and the date of onset of the event. Since they were age-ineligible for the vaccination, they are only considered for the indirect analysis. For analyses of vaccine indirect protection against illnesses (blood culture proven *S.* typhi episodes, blood culture proven *S.* paratyphi episodes, and persistent fever episodes), we included only episodes whose onsets occurred after their dates of entry. For those who reached the age beyond the surveillance age (age >18 years), we defined that point as exit date, and we excluded those illnesses that were reported after the exit date. For all other events noted below, we included all events whose onsets were between entry date and exit date. Follow-up time was also calculated from date of entry.

	Age less than 2 years at *ZT*	**Age greater than 16 and less than 18 years at *ZT***
Date of entry	The date reaching the age 2 years during the follow up period.	The median date of vaccination of the person's assigned cluster.
Maximum date of exit (may be censored earlier)	The median date of vaccination of the person's assigned cluster + 730 days.	The date the person reaches 18 years of age.
Onset of illness	≥ Date of entry + 1 day	≥ Date of entry+ 1 day
Date of migration out	≥ Date of entry	≥ Date of entry
Date of death	≥ Date of entry	≥ Date of entry
Beginning of follow-up	Date of entry	Date of entry *(=Zero time*)

11. Principal analyses

Analyses of protection by Vi polysaccharide vaccine: These analyses considered total, indirect, and overall protection. Outcomes for these analyses included both those for which protection by Vi was anticipated (*S.* typhi episodes, and episodes of persistent fever) as well as against *S.* paratyphi episodes, for which protection by Vi was not anticipated. The latter outcome was analyzed to evaluate the possibility of bias in the trial (reflected by protection against *S.* paratyphi episodes by Vi polysaccharide), as well as to evaluate whether immunization by Vi is associated with a replacement of typhoid fever by paratyphoid fever (reflected by an elevated risk of *S.* paratyphi episodes associated with Vi polysaccharide).

Total protection

Total protection is estimated by comparing the incidence of the outcome among Vi recipients and the incidence of the outcome among Hepatitis A vaccine recipients. It reflects the combination of direct protection of vaccinees by Vi together with herd protection of vaccinees in Vi-vaccinated clusters. For each type of event considered in primary analyses, we focused only on the initial event experienced by an individual.

Numerator episodes for analysis:

1. Culture positive typhoid episodes
Culture positive typhoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day and *zero time* plus 730 days (unless earlier censored as described below for the denominator). We excluded episodes where identity check failed to confirm the visit to project health clinic for febrile illness.

2. Culture positive paratyphoid episodes
Culture positive paratyphoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day and *zero time* plus 730 days unless earlier censored as described below for the denominator. We excluded episodes where identity check failed to confirm the visit to project health clinic for febrile illness.

3. Persistent fever episodes
Persistent fever episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day and *zero time* plus 730 days unless earlier censored as described below for the denominator.

Denominator for analysis:

Subjects in the denominator for analysis are those who were age- and otherwise eligible to receive a vaccine and were noted as having received either Vi or Hepatitis A vaccine. Subjects noted as having been vaccinated but whose date of exit was ascertained to be before vaccination (*vida supra*, see appendix) are excluded. In analyses of time-to-event, the person-time at risk begins at *zero time* and continues until the date of death, date of out-migration, date of internal migration, date of event under analysis which ever comes first; otherwise, *zero time* plus 730 days

Indirect protection
Indirect protection is estimated by comparing the incidence of the outcome among non-recipients of Vi in the Vi clusters and the incidence of the outcome among non-recipients of Hepatitis A vaccine in the Hepatitis A vaccine clusters. It reflects the herd protection of non-vaccinees in Vi-vaccinated clusters. For each type of event considered in primary analyses, we focused only on the initial event experienced by an individual.

Indirect protection analysis (a)

We considered only those who were available and age- and residency- eligible for vaccination at *zero time*.

Numerator episodes for analysis:

1. Culture positive typhoid episodes
Culture positive typhoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator). We excluded episodes where identity check failed to confirm the visit to project health clinic for febrile illness.

2. Culture positive paratyphoid episodes

Culture positive paratyphoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator). We excluded episodes where identity check failed to confirm the visit to project health clinic for febrile illness.

3. Persistent fever episodes
Persistent fever episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days unless earlier censored as described below for the denominator.

Denominator for analysis:

Subjects in the denominator for analysis will be those who were noted as having received neither Vi nor Hepatitis A vaccine but were age-eligible for vaccination and residents of the study clusters at *zero time*. In analyses of time-to-event, the person-time at risk begins at *zero time*, and continues until the date of death, date of out-migration, date of internal migration, date of event under analysis which ever comes first; otherwise, *zero time* plus 730 days.

Indirect protection analysis (b)

We considered those who were available at *zero time*, were not eligible for vaccination, but were age- and residency- eligible for surveillance (2 to 18 years of age at any time during the follow up period).

Numerator episodes for analysis:

1. Culture positive typhoid episodes
Culture positive typhoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator) for those who were age- and residency- eligible for vaccination at *zero time*. For others (age- and residency- eligible for surveillance but not for vaccination at *zero time*), the onsets of such episodes must have been between entry date plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator). We excluded episodes where identity check failed to confirm the visit to project health clinic for febrile illness.

2. Culture positive paratyphoid episodes
Culture positive paratyphoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator) for those who were age- and residency- eligible for vaccination at *zero time*. For others (age- and residency- eligible for surveillance but not for vaccination at *zero time*), the onsets of such episodes must have been between entry date plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator). We excluded episodes where identity check failed to confirm the visit to project health clinic for febrile illness.

3. Persistent fever episodes
Persistent fever episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator) for those who were age- and residency- eligible for vaccination at *zero time*. For others (age- and residency- eligible for surveillance but not for vaccination at *zero time*), the onsets of such episodes must have been between entry date plus one day until *zero time* plus 730 days (unless earlier censored as described below for the denominator).

Denominator for analysis:

Subjects in the denominator for analysis will be those who were noted as having received neither Vi nor Hepatitis A vaccine but were age-eligible for surveillance and residents of the study clusters at *zero time*. In analyses of time-to-event, the person-time at risk begins at date of entry or *zero time*, and continues until the date of death, date of out-migration, date of internal migration, date of event under analysis which ever comes first; otherwise, *zero time* plus 730 days.

Overall protection

Overall protection is estimated by comparing the incidence of the outcome among population in the Vi clusters at *zero time* and the incidence of the outcome among population in the Hepatitis A vaccine clusters at *zero time*. It reflects the protection of the general population regardless of vaccine uptake.

Numerator episodes for analysis:

1. Culture positive typhoid episodes

Culture positive typhoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days unless earlier censored as described below for the denominator. We excluded episodes where identity check failed to confirm the visit to project health clinic for febrile illness.

2. Culture positive paratyphoid episodes

Culture positive paratyphoid episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days unless earlier censored as described below for the denominator. We excluded episodes where identity check failed to confirm the visit to project health clinic for febrile illness.

3. Persistent fever episodes

Persistent fever episodes are defined above. To be included in the analysis, the onsets of such episodes must have been between *zero time* plus one day until *zero time* plus 730 days unless earlier censored as described below for the denominator.

Denominator for analysis:

Subjects in the denominator for analysis will be all those age- and residency- eligible persons present at *zero time.* The person-time at risk begins at *zero time* and continues until the date of death, date of out-migration, date of internal migration, date of event under analysis which ever comes first; otherwise, *zero time* plus 730 days

Analysis of safety

Surveillance for safety consisted of the following:

I. Observation 15-30 minutes after vaccination

All vaccine recipients were asked to remain at the vaccination site for 30 minutes immediately after vaccination, and all local and systemic adverse events were noted.

II. Adverse events 3 days after vaccination

A sub-sample of 480 individuals was listed to be visited at home daily for 3 days after vaccination. At each visit, all adverse events observed by the trained study personnel or reported by the parents/guardians of the subject were recorded.

III. Other adverse events in the month after vaccination

Vaccinees were asked to attend designated project health facilities including all hospitals serving the study population if local or systemic events occurred during the month after immunization. A special study form was completed for every observed case, and a senior study physician made an assessment of causal relationship between receipt of the study vaccine and the occurrence of the adverse event.

IV. Surveillance of deaths in the first month following vaccination

All deaths among trial participants during the follow-up period were identified through weekly household visit as part of the augmented passive disease surveillance, through the health facilities, and/or censuses carried out after vaccination. A special study verbal autopsy form was completed for every death.

Separate analyses will be conducted for each of these four categories of events. In each of these analyses, the risk of the cited event will be compared for recipients of Vi vaccine versus recipients of Hepatitis A vaccine. Events will be categorized as severe or non-severe using standard ICH-GCP definitions. All events will be analyzed, regardless of the physicians' judgments about causality. For all categories of events, events whose onset occurred on or after *zero time* will be considered. For categories I, III and IV, the denominator will be the entire vaccinated population at risk. For category II, the denominator will be the sub-sample of vaccinees who were followed up at home.

Analysis of immune responses

A sub-sample of vaccinees was bled immediately before vaccination, 42 days after *zero time*, and again at two years after *zero time*. Sera from these samples were assessed for IgG anti-Vi antibody titers and antibodies against Hepatitis A (anti-HAV) at GSK laboratories in Rixensart, Belgium, blinded to the identity of the vaccine that each subject had received.

Analyses compare recipients of Vi and Hepatitis A vaccine for geometric mean titers (GMT) and the proportion with protective titers at each bleed, and for the proportions seroconverting between baseline and bleed 2, and between baseline and bleed 3.

12. Statistical analyses

Analysis of vaccine effectiveness

Comparisons of individual-level baseline and adverse event variables will employ generalized estimating equations (GEE) to adjust for membership in the randomized clusters, with the logit link function for dichotomous variables and the identity link function for dimensional variables. Cluster-level variables are compared using the Student t-test for dimensional variables and the chi-square test for categorical variables.

The primary objective of the study is to evaluate *total vaccine protection*, comparing rates of episodes of typhoid fever in recipients of Vi vaccine versus recipients of hepatitis A vaccine. As secondary objectives, we will estimate the *indirect vaccine protection* by comparing the rates of typhoid fever in non-vaccinees in the Vi clusters versus those in the hepatitis A vaccine clusters, and *overall vaccine protection* by comparing rates of typhoid fever among all residents of the Vi clusters versus the hepatitis A clusters. Finally, to assess whether bias could have distorted the rates of typhoid fever in the Vi vaccine versus the hepatitis A vaccine clusters, we will evaluate total protection by Vi vaccine against episodes of paratyphoid fever (all of which were due to *S. paratyphi* A in our study), a disease for which protection by Vi vaccine is not anticipated but which has clinical characteristics closely resembling typhoid fever. In all analyses, we will evaluate vaccine protection against first episodes of the disease outcome whose onsets occurred on or after the day following zero-time.

Analyses of vaccine protection will use Cox proportional hazard regression models, verifying first that the proportionality assumption is satisfied for all independent variables. Hazard ratios (HRs) of the target outcome in the Vi versus the hepatitis A groups are estimated by exponentiating the coefficient for the vaccine variable in these models, and vaccine protective effectiveness (PE) is estimated as [(1- HR) X 100%]. Standard errors for the coefficients are used to estimate P values and 95% confidence intervals for the HRs. Simple analyses of vaccine impact are adjusted for the design effect of the cluster randomization using robust sandwich variance estimates. Final adjusted estimates are obtained from models that also included the variables used for stratified randomization, as well as variables which are found to be independently associated with the time to the event at P<.1 in a backward selection algorithm. To evaluate heterogeneity of vaccine protection among different subgroups, we will evaluate interaction terms between the vaccination and subgroup variables in these models. All P values and 95% confidence intervals for these analyses are calculated as two-tailed.

Analysis of vaccine safety

Evaluation of vaccine safety entailed comparison of recipients of Vi vaccine with those of hepatitis A vaccine for immediate adverse events, for events ascertained daily for three days after dosing in a subset of participants, for medical events detected in study clinics and hospitals during the month after dosing, and for mortality, including cause-specific mortality, during the month following dosing. Statistical evaluation of these outcomes, which were dichotomous, employed Generalized Estimating Equations, with the logit link function to adjust for cluster allocation.

P value thresholds
A P value less than 0.05 (two-tailed) is considered statistically significant for all analyses.

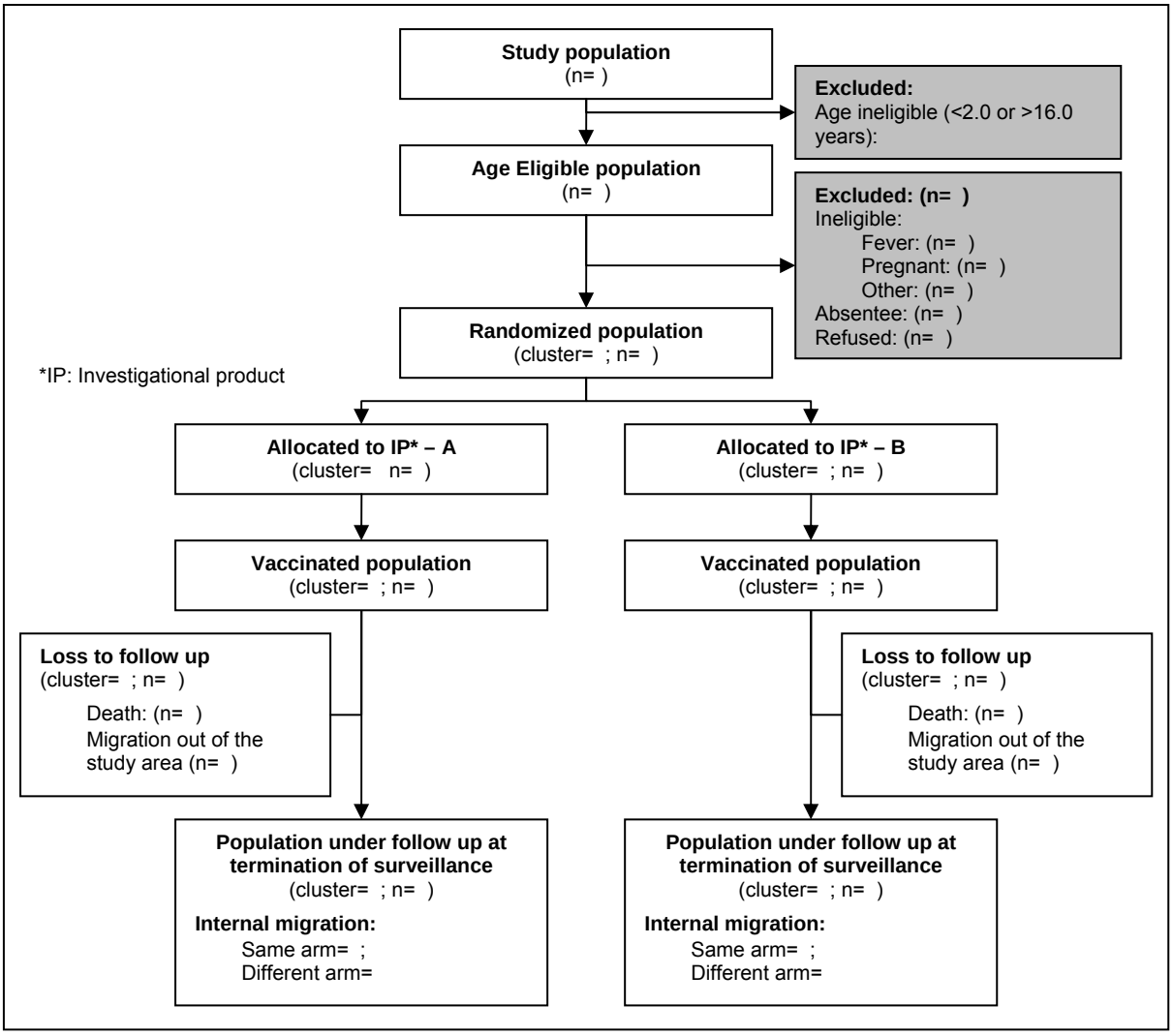
13. Analysis specifics

Prior to unblinding of the codes for the trial, all analyses will have been conducted on a "re-randomized data set", in which the 120 clusters for the trial have been randomly reassigned to 2 groups, each with 60 clusters. This was done to facilitate development of an analysis plan in a fashion that was not influenced by knowledge of which vaccine had been received, and also that allowed creation of the complete ensemble of analytic programs prior to unblinding.

Assembly of persons and events
Contemporary medical journals require that papers conform to the guidelines in the Consolidated Standards of Reporting Trials (CONSORT) statement. The statement includes a checklist of items that should be included in the trial report. In addition, the CONSORT statement recommends including a flow diagram to show the flow of participants from group assignment through to the final analysis. The special flow diagram proposed for denominators for cluster-randomized trials has been proposed, and we have adapted the diagram for this trial. In addition, we have developed a corresponding flow diagram for numerator events under analysis.

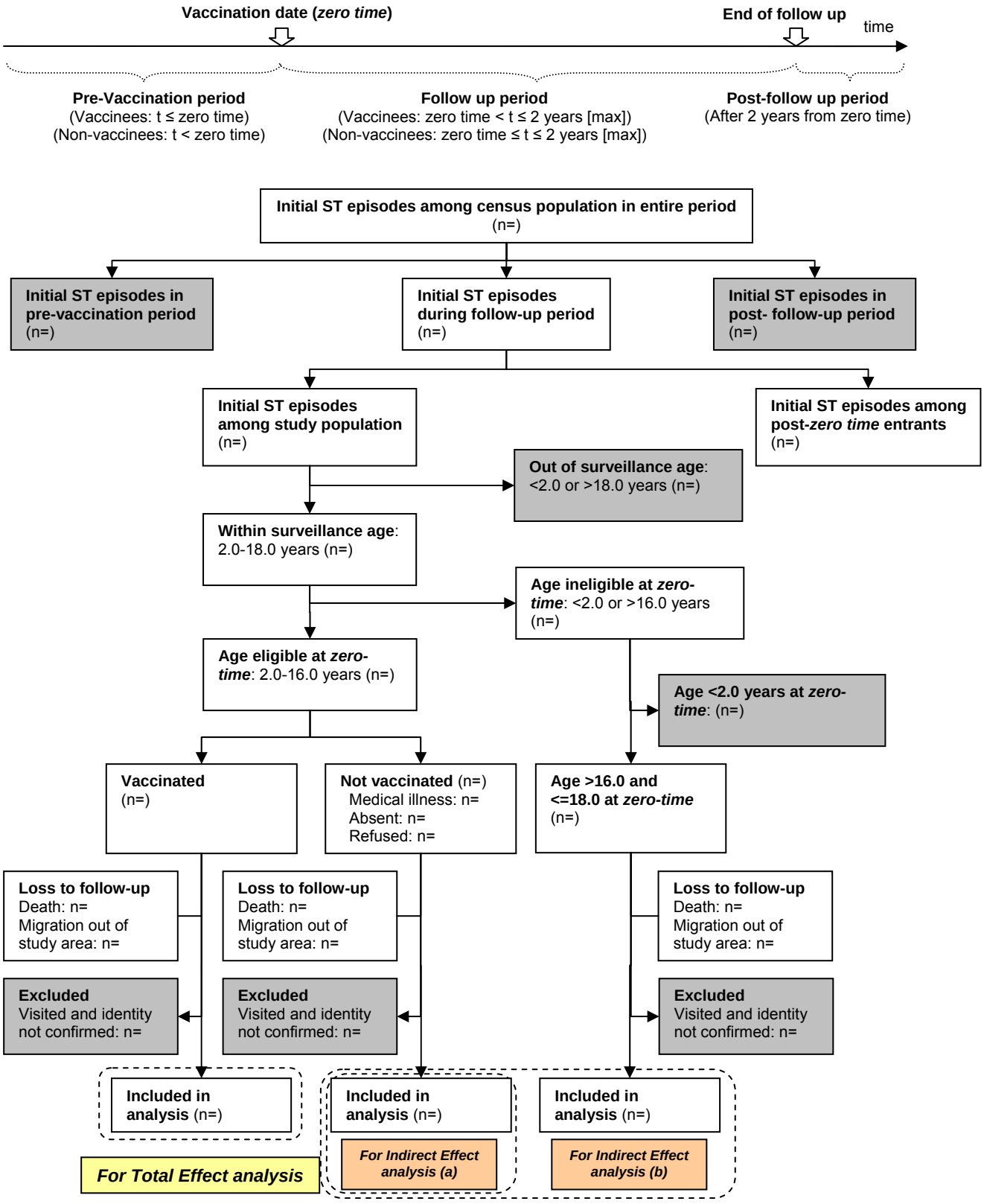
1. CONSORT chart for denominators

Above chart represents the flow of study population from selection and randomization to final follow up showing the loss to follow up.



2. Case flow charts for numerators

In contrast to the CONSORT chart for the denominators, the case flow chart shows the flow of cases (numerators) detected during the whole study period as above. This is prepared for all outcomes of interests: culture-confirmed *S.* typhi, *S.* paratyphi, and persistent fever.



Candidate zero time variables for inclusion as covariates in final multivariable, Cox regression models of Vi vaccine protection

Candidate *zero time* variables for inclusion as covariates in final multivariable, Cox regression models of Vi vaccine protection are listed below. Note that if several of variables measure the same construct (e.g. socio-economic status, status of drinking water and hygiene), we selected only the variable most strongly associated with the outcome for the model.

Candidate variables and their categories:

Variable	**Categories**
Age	In years (continuous)
Age Group	0: <5 years 1: 5 years and above
Gender	0: female 1: male
Individuals living in a household with household head having any education	0: No 1: Yes (any type of education)
Individuals living in a household using safe toilet	0: others 1: Safe toilet: use of own (non-shared) flush toilet
Individuals living in a household using safe water source	0: No 1: Yes (tap at household level)
Individuals living in owned house	0: No 1: Yes
Individuals living in a household owning refrigerator	0: No 1: Yes
Individuals living in a household owning motorbike	0: No 1: Yes
Individuals living in a household owning washing machine	0: No 1: Yes
Individuals living in a household owning at least one luxury item	0: No 1: Yes; items: Refrigerator, motorbike, and/or washing machine
Monthly household income	In Pakistani Rupee (continuous)
Individuals with high monthly household income	0: On/Below median household monthly income 1: Above median household monthly income
Per-capita monthly income	In Pakistani Rupee (continuous)
Individuals with high per-capita monthly income	0: On/Below median household per-capita monthly income 1: Above median household per-capita monthly income
Cluster size	No. of age eligible individuals (continuous)
Individuals living in larger cluster specified in the stratification	0: On/Below median cluster size 1: Above median cluster size
Household size	No. of total individual (continuous)
Number of household in the cluster	No. of households (continuous)
Population density /10m^2	Total study population/area (continuous)
Higher population density /10m^2	0: On/Below median population density 1: Above median population density
Distance (m) from the household to the project health center	Distance in meters (continuous)
Longer distance from the household to the project health center	0: On/Below median distance to the health center 1: Above median distance to the health center
Pre-vaccination incidence of typhoid fever	Continuous

Pre-vaccination incidence of typhoid fever	0: On/Below median pre-vaccination typhoid incidence 1: Above median pre-vaccination typhoid incidence
Residence: Sultanabad vs. Bilal	0: Sultanabad 1: Bilal
Residence: Hijrat Colony vs. Bilal	0: Hijrat 1: Bilal
Vaccination coverage	Continuous

Subgroup analyses:
We pre-specified several zero time and post-zero time variables and their categories for subgroup analyses of vaccine protection. The pre-specified variables and their categories are given below.

Variables and their categories used for subgroup analyses:
These variables were selected based on aforementioned criteria using the re-randomized data

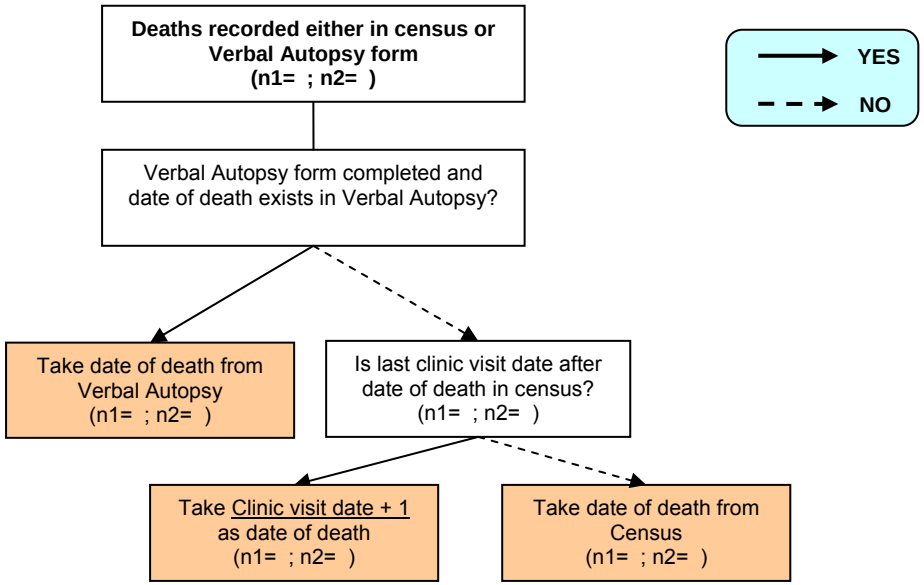
Variable	**Categories**
Age at zero time	0. 2-4.9 years 1. 5 years and above
Gender	0. Female 1. Male
Socio-economic status (SES)	0. Below median per-capita household monthly income 1. On/Above median per-capita household monthly income
Safe water source	0. Households without safe water 1. Households with safe water
Population density	0. Below median cluster population density 1. On/Above median cluster population density
Pre-vaccination incidence of typhoid fever for the cluster	0. Below median pre-vaccination typhoid incidence 1. On/above median pre-vaccination typhoid incidence
Vaccination coverage	0. First tertile of coverage of the entire cluster by either vaccine 1. Second tertile of coverage of the entire cluster by either vaccine 2. Third tertile of coverage of the entire cluster by either vaccine
Site	0. Bilal Colony 1. Sultanabad/Hijrat Colony
Year of follow-up	0. Onset between zero time (ZT) and ZT+365 1. Onset between ZT+366 and ZT+730

APPENDICES

1. Demographic date assignment flowchart
2. Salmonella Typhi specimens for the analysis of Vi vaccine effectiveness

APPENDIX 1: Demographic date assignment flowchart for KARACHI TYPHOID TRIAL

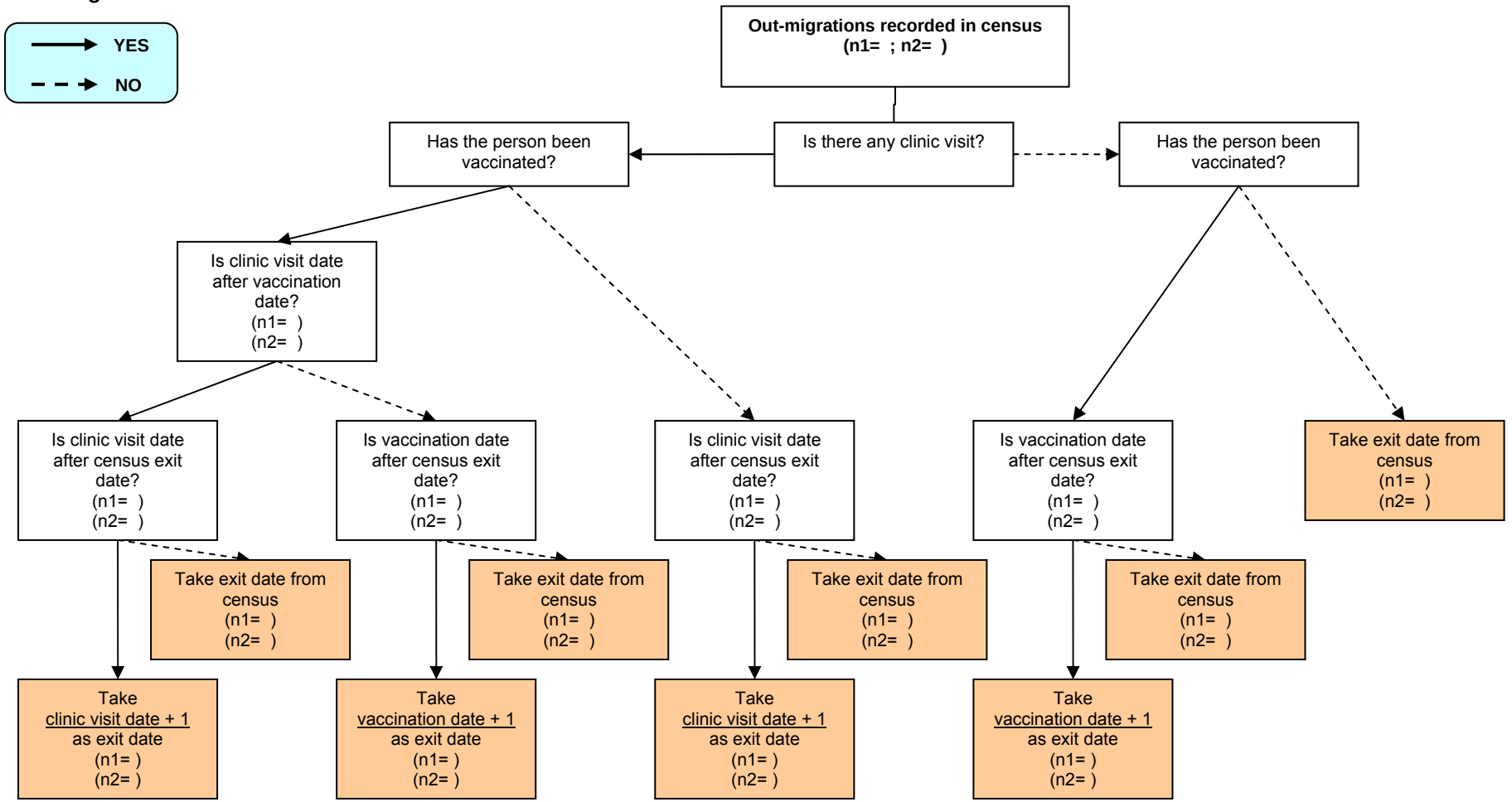
1. Deaths



Footnote:

- n1 is the number from whole period
- n2 is the number from at or after zero time
- We do not consider vaccination status in adjusting the date of death, since it would arbitrary place the death to the day after receiving the vaccine
- Reclassification of date of death can only move the date forward in time or stay the same

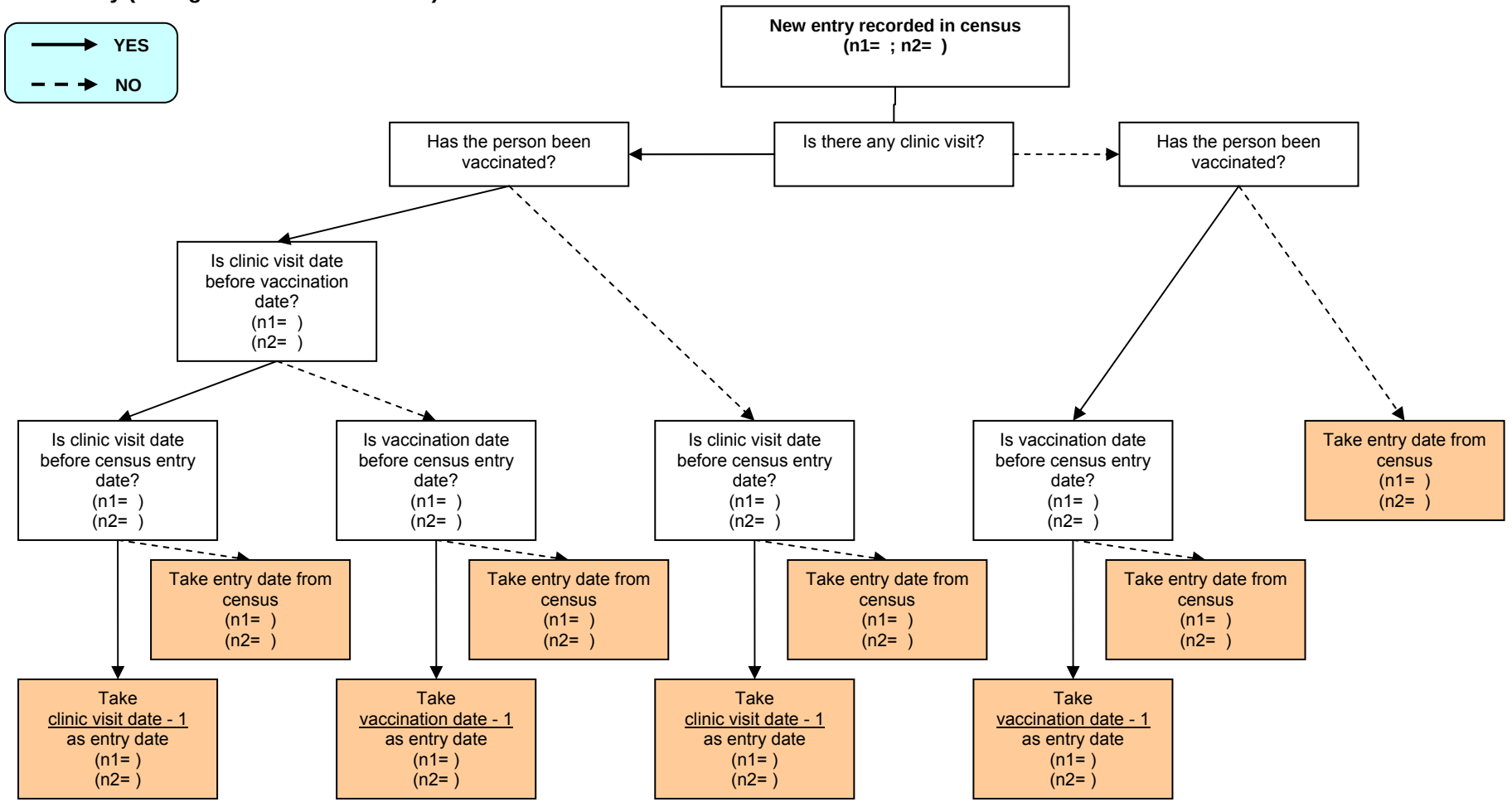
2. Out-migrations



Footnote:

- n1 is the number from whole period
- n2 is the number from at or after zero time
- Reclassification of date of migration out can only move the date forward in time or stay the same

3. New entry (In-migrations and newborns)



Footnote:

- New entry includes both in-migration and being born
- n1 is the number from whole period
- n2 is the number from at or after zero time
- Reclassification of date of entry can only move the date backward in time or stay the same

APPENDIX 2

Salmonella Typhi specimens for the analysis of Vi vaccine effectiveness (Karachi)

1. **Collection and handling at the site**

Blood specimens were collected in the field health centers (project ones and private clinics). After all relevant information were recorded in project forms, they were transferred to the Aga Khan University Hospital Clinical Laboratory (AKUHCL) Pick-up Points. They were then transported to the AKUHCL for testing. AKUHCL is a general laboratory taking care of specimens from AKU hospital, pick-up points, as well as from other collaborating institutions.

Field sites were visited frequently (approximately every month) by IVI staff to check on these activities.

2. **Assessment at the site**

The assessment was done at the AKUHCL. The method is standardized and was done for not only the study materials but also regular specimens requested by the physicians for laboratory diagnosis. Details on microbiological assessment are described in the protocol.

Laboratory was visited once to twice a year by an IVI Microbiology Task Force member.

3. **Storage of the isolates at local site**

Isolates are kept in -70°C freezer in the AKUHCL. These are called IVI isolates and kept separately from other specimens. The isolates are stored by inoculating a thick suspension of growth in nutrient broth with 15% glycerol in cryovials. Each tube is labeled with the tube number, L-number (AKUHCL unique specimen code) and the name of the patient.

They were checked during the visits by the IVI Microbiology Task Force member.

4. **Reference lab at the Ho Chi Minh City**

A part of the specimens from pre-vaccination period was shipped to the reference lab (Oxford University Clinical Research Unit, HCMC, Vietnam) for confirmation.

Oxford University Clinical research Unit has used API 20E for biochemical confirmation and polyclonal and monoclonal antisera for serology. API 20E method allows identification of the strains according to 20 biochemical characteristics. The API galleries are standard tests that show reproducible results in different lab or settings.

Initial shipment (first quarter 2003) of 53 specimens had shown wide discrepancies (35/53=66% discrepancy) in reported outcomes.

AKU – results based on project logbook
OX – results based on Oxford Unit

	AKU ST	**AKU SPA**	**AKU SSP**	**Total**
OX ST	14	0	0	**14**
OX SPA	3	4	0	**7**
OX SSP	24	8	0	**32**
Total	**41**	**12**	**0**	**53**

IVI microbiology task force (Anne-Laure Page and John Wain) had visited AKU for investigation in July 2003 and taken 8 specimens for retesting at AKU. The results reported by Oxford Unit were different, which suggested that the organisms analyzed at Oxford Unit were different from the

organisms stored at AKU. The reasons for these differences could not be found. The problems could be either at the AKU lab, somewhere during the shipment or upon reception at Oxford Unit.

We re-packed the specimens and re-sent to Oxford unit for analysis (third quarter 2003). The AKU and Oxford results this time were concordant except for 4 specimens (4/53), where AKU had identified as typhi, but Oxford Unit had identified as non-typhi salmonella species (3) and one could not be grown.

AKU – results based on project logbook
OX – results based on Oxford Unit

	AKU ST	AKU SPA	AKU SSP	Total
OX ST	37	0	0	**37**
OX SPA	0	12	0	**12**
OX SSP	3	0	0	**3**
Total	**40**	**12**	**0**	**52**

In summary, initial shipment was contaminated and the second shipment had shown 94% concordance (49/52).

5. Reference lab at the IVI and the KNIH

All specimens from pre- and post-vaccination period were shipped to the IVI for banking and re-confirmation purposes. 768 specimens were sent from AKU to IVI in August 2007. The AKU laboratory results (project logbook) of the specimens are as following:

Species	Number
S. Typhi	524
S. Paratyphi A	220
S. Paratyphi B	8
Other Salmonella	15
Non-Salmonella	1

Our focus was the specimens from the trial follow-up period – 262 samples.

IVI lab has decided to use the PCR method for confirmation of the specimens. Though the "gold standard" remains microbiological confirmation of the organism, the molecular confirmation (PCR) has been extensively studied and shown higher specificities than microbiological methods. The PCR method is now validated and used where facilities are available. One of the major strength of PCR is the relatively rapid assessment of the specimens.

Confirmation plan:

1. multiplex PCR – this is to determine typhi or non-typhi

   The multiplex PCR (mPCR) included 5 primer pairs for identification of typhi:

Primer	Characteristics
InvA	Identify salmonella
ViaB	Identify Vi gene
Dh	Identify flagella d antigen
Prt	Identify Group D O antigen
IAC	Positive control

2. sequencing for those which are non-typhi by mPCR – this is to identify other salmonella

   Those that are negative to the mPCR had then been sequenced for the aroC gene. The aroC gene is used for MLST analysis of salmonella. Salmonella species can be distinguished depending on the allele types:

Allele	Species
1	Typhi
5	Dublin/Enteriditis/St. Paul
10	Typhimurium/Newport
45	Paratyphi

The aroC sequencing region is 501 nucleotides, allele type 5 and 45 are different from allele type 1 at 7 nucleotide position, and allele type 10 is different from allele type 1 at 5 nucleotide position. There is very low possibility that this allele type analysis can give false result by the sequencing error.

3. simplex PCR for those which are negative to both mPCR and sequencing – since simplex PCR is more sensitive than multiplex PCR, this was done to identify any possible non-identification due to the PCR method (safe guarding) [*Note that none underwent this step*]

4. microbiological method (the same way as AKU) for those which are non-typhi by PCR together with positive controls at KNIH (Salmonella reference lab for Korea)

Results:

Table 1: Comparison of results from IVI (PCR) and AKU

AKU – results based on project logbook
IVI – results based on IVI

	AKU ST	AKU SPA	AKU SPB	AKU SSP	Total
IVI ST	124	4	0	0	**128**
IVI non-ST	19	107	3	5	**134**
Total	**143**	**111**	**3**	**5**	**262**

→ S. Typhi concordance rate was 87% (124/143)

Table 2: Comparison of results from IVI (PCR) and KNIH (microbiology)

Note that all discordant specimens and random selection of concordant specimens from Table 1 were sent to KNIH for microbiological assessment.

KNIH – results based on KNIH
IVI – results based on IVI

	KNIH ST	KNIH SPA	KNIH SPB	KNIH SSP	Total
IVI ST	34	0	0	0	**34**
IVI non-ST	0	115	3	16	**134**
Total	**34**	**115**	**3**	**16**	**168**

→ Concordance rate was 100% (168/168)

Table 3: Comparison of results from KNIH and AKU

AKU – results based on project logbook
KNIH – results based on KNIH

	AKU ST	AKU SPA	AKU SPB	AKU SSP	Total
KNIH ST	30	4	0	0	**34**
KNIH SPA	11	104	0	0	**115**
KNIH SPB	0	1	2	0	**3**
KNIH SSP	8	2	1	5	**16**
Total	**49**	**111**	**3**	**5**	**168**

→ Concordance rate was 84% [(30+104+2+5)/168]

Table 4: Comparison of results from Reference lab (IVI and KNIH combined) and AKU logbook

Based on the result from Table 2 for PCR being perfectly concordant with microbiological method at KNIH, we consider PCR results OR microbiology results at KNIH as reference lab results.

AKU – results based on project logbook
Ref – results based on IVI or KNIH

	AKU ST	AKU SPA	AKU SPB	AKU SSP	Total
Ref ST	124	4	0	0	**128**
Ref SPA	11	104	0	0	**115**
Ref SPB	0	1	2	0	**3**
Ref SSP	8	2	1	5	**16**
Total	**143**	**111**	**3**	**5**	**262**

→ Concordance rate was 90% [(124+104+2+5)/262]

6. Retesting of samples at local lab and at IVI lab

Due to the similar discrepancies (non-typhoidal salmonella) seen previously from Oxford lab, we decided to re-test the specimens locally, assuming that there had been contamination during subculture or transport. A visit was made in February 2008 to AKU and Leon has observed the AKU laboratory staff for this activity.

Samples chosen (results from KNIH/IVI were blinded to the lab technician):

27 discrepant samples between AKU and KNIH/IVI (shaded pink in Table 4 above)
47 concordant samples between AKU and KNIH/IVI from above in random selection
1 sample which did not grow at KNIH/IVI
Total – 75 samples for re-testing

Results:
Checking the lab result book and project logbook

- We found the sample 525 is incorrectly entered as ST in the project logbook, while it was actually written S. Typhimurium in the lab result book. We corrected the project logbook.
- We found the sample 589 is incorrectly entered as Sal. Group B in the project logbook, while it was actually written Sal. Group D in the lab result book. We corrected the project logbook.
- We found tubes 708 and 709 were labeled incorrectly – this was found since the name and the tube number did not match with the lab result book.
    - Information in the lab result book: tube serial number, lab number, name, lab result, etc.
    - Information on the tube: tube serial number, lab number, name
    - *Since lab number and name are always together to process the specimens at AKU lab, we considered this information relates to the lab results, which was noted in the project logbook. The tube serial number, thus, was likely to have been incorrectly written when cataloguing for storage, which is a process that is only done when the lab technician is handling IVI/DOMI specimens.*

AKU log	Lab no.	AKU Result	Tube no.	IVI result	KNIH result
708	697563	SPA	709	Non-ST	SSP
709	706777	ST	708	ST	ST

    - We therefore corrected the numbers on the tube.

Table 4b: Comparison of results from Reference lab (IVI and KNIH combined) and AKU (project logbook) after correcting the errors in the logbook

AKU – results based on project log book
Ref – results based on IVI or KNIH

	AKU ST	AKU SPA	AKU SPB	AKU SSP	Total
Ref ST	124+1[708]	3	0	0	**128**
Ref SPA	11	104	0	0	**115**
Ref SPB	0	1	2	0	**3**
Ref SSP	6	2+1[709]	1	5+1[525]	**16**
Total	**142**	**111**	**3**	**6**	**262**

Chosen samples after correcting the project logbook:
25 discrepant samples between AKU and KNIH/IVI (shaded orange in Table 4b above)
49 concordant samples between AKU and KNIH/IVI from above in random selection
1 sample which did not grow at KNIH/IVI
Total – 75 samples for re-testing

Subculture and biochemical assessment

- These 75 samples were taken from the freezer (mother stock) and subcultured
- One specimen (sample 176) had very little growth, but all other grew fine
- API-20E and serological assessments were done

Table 5 – Comparing the results from Reference lab (IVI and KNIH combined) and re-testing by API-20E and Serology

Retest – results based on retesting at AKU
Ref – results based on IVI or KNIH

	Retest ST	Retest SPA*	Retest Grp B	Retest Grp D	Total
Ref ST	26	0	2	0	**28**
Ref SPA	11	21	0	0	**32**
Ref SPB	1	0	0	0	**1**
Ref SSP	5	2	3	3	**13**
Total	**43**	**23**	**5**	**3**	**74**

* 1 did not grow at Reference Lab

→ Concordance rate was 72% [(26+21+3+3)/74]

Table 6 – Comparing the results from AKU (project logbook) and re-testing by API-20E and Serology

Retest – results based on retesting at AKU
AKU – results based on project log book

	Retest ST	Retest SPA	Retest Grp B	Retest Grp D	Total
AKU ST	41	0	0	1[421]	**42**
AKU SPA	3 [115, 176, 559]	24	2[184, 709]	0	**29**
AKU SPB	0	0	1	0	**1**
AKU SSP	0	0	1[Typhimurium]	2	**3**
Total	**44**	**24**	**4**	**3**	**75**

→ Concordance rate was 92% [(41+24+1+1+2)/75]

Table 7 – Discordant results from Table 6

Tube Number	AKU logbook	Initial API-20E	Initial Ref Lab	Retest at AKU	Retest at AKU API-20E	Retest at IVI	Interpretation

115	SPA	n/a	ST	ST	4404540	ST	Initial API-20E result unknown: it could be that there was a recording mistake at initial stage
176	SPA	0104552	SPB	ST	4404540	SPP (SPB)	Clone growth was skeptical: possibly loss of mother stock.
184	SPA	0104552	ST	Grp B	6704552	SPP (SPB)	Mistake in initial identification? Wrong specimen sent initially?
421	ST	4704550	Dublin	Grp D (Dublin)	6704550	SPP (Grp D)	API-20E=4704550 is not ST; it is Group D: a transcription mistake at initial stage
559	SPA	0104552	ST	ST	4404540	ST	Mistake in initial identification?
708 → 709	SPA	0104552	Typhimu rium	Grp B	6704552	SPP (Grp B)	Tube labeling was incorrect (708 and 709)*. Typhimurium is Group B. Mistake in initial identification?

***** This was found since the name and the tube number on the tube did not match with the logbook

75 specimens from the retesting were packed and sent to IVI in March for final confirmation. They were retested at IVI using PCR method. 74 samples matched with AKU retesting result, one that did not match was the tube 176, which from the initial subculturing, the growth was very slow and skeptical for being the original stock.

Table 8 – Comparing the results from re-testing at AKU and IVI/KNIH

Retest – results based on retesting at AKU
reIVI – results based on retesting at IVI/KNIH

	Retest ST	Retest SPA	Retest Grp B	Retest Grp D	Total
reIVI ST	43	0	0	0	**43**
reIVI SPA	0	24	0	0	**24**
reIVI Grp B	1 [176]	0	4	0	**5**
reIVI Grp D	0	0	0	3	**3**
Total	**44**	**24**	**4**	**3**	**75**

→ Concordant rate was 99% (74/75)

Summary of retesting:

From the retesting of the selected 75 specimens, the results were concordant between AKU and IVI except for one. The discordant one (tube 176) had a very slow growth when subcultured at AKU, and likely that the mother stock is lost. For the other 74 specimens, they were consistent. The 6 discordant ones from the AKU (project logbook) (Table 3), at least 2 of them (shaded in table 4) were likely to be due to the initial transcription mistake.

Based on biochemical and serology tests, there were three (3) Group D specimens which are *Salmonella* Dublin (Factor 9 positive and Hp positive). We were initially worried that there was a contamination of unknown origin. However, this finding suggests contamination had occurred from our own specimens. Interestingly, *S.* Dublin has Vi, so in principle, Vi vaccine can protect ones with *S.* Dublin infection.

It was also found that most of the mother stocks seem to have been conserved (comparing the initial API-20E results and new API-20E results from this re-testing).

7. Preparation for the analysis

We can conclude that the initial assessments were correct based on the observation of the lab's practice and the results of the retesting done previously (with Anne-Laure and John Wain's presence) and recently (with Leon's presence). Assuming the reference lab results are correct and the discrepancies were not due to loss of mother stock, 4 discrepancies (after subtracting 2 transcription errors) out of 75 retested is error rate of **5% (95% concordance)**, which is in the acceptable range for a clinical lab. (For reference lab, the error rate should be less than 5%; for clinical lab, less than 10%)

* * *