Final Report

India Hib Vaccine Probe Study: Part A

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Introduction:

Haemophilus influenzae has long been recognised as a cause of serious infections (i.e. pneumonia and meningitis) and mortality in children less than 5 years old. Prior to the introduction of *haemophilus influenzae* type b (Hib) immunization, the incidence of invasive *Hib* infection was 20–50/100,000 children <5 years of age in industrialized countries and higher in developing regions. Hib disease has been virtually eliminated in a number of developing countries where Hib vaccine has been introduced into the routine immunization programmes.

Hospital-based information from India showed that Hib was the most common cause of bacterial meningitis in children under age 5 years. Establishing the etiology of pneumonia is more difficult than meningitis. The proportion of cases of lobar pneumonia caused by Hib has been estimated to vary from 16-63%. Vaccine probe studies i.e., a study design where estimation of burden of vaccine preventable Hib disease is done by measuring the proportion of pneumonia and meningitis cases prevented during clinical trials of Hib conjugate vaccine, represent an important tool for estimating this undiagnosed disease burden. In 2004, to augment Hib incidence data from Asia and to support an evidence-based decision for Hib vaccine introduction in India, the Government of India (GoI) decided to conduct a Hib vaccine probe study in three geographically diverse sites. This probe study was intended to be implemented in two parts: a preparatory phase and the randomized double blind controlled phase. The data presented here is from the preparatory phase, the purpose of which was to determine the feasibility of conducting a randomized vaccine probe study at these sites and to determine the incidence of all cause pneumonia and meningitis in order to calculate the sample size for the probe study. In 2006, the World Health Organization urged countries to move forward with Hib vaccine introduction at national level. Therefore, it was not considered ethical to proceed with a prospective randomized trial.

This study was funded by The Global Alliance for Vaccines and Immunization (GAVI) Hib Initiative, USAID India, and Indian Council of Medical Research, Delhi, India. Ethical clearances from each sites' Institutional Review Board, along with clearances from the GoI and Johns Hopkins University, were obtained. Prior to enrollment in community or hospital written consent was obtained from a parent of each study participant.

Methods

The preliminary phase of the study had three components: a cohort study, a hospital based surveillance of pneumonia and meningitis and Hib carriage study. The methods for each component are listed below.

Cohort Study

The objective of the cohort study was to estimate the incidence of hospitalized pneumonia and meningitis among children less than 2 years of age in India, in the absence of Hib vaccination. Each participating institution selected a rural development block (approximately 100,000 total population) as a study area, under the assumption that private sector Hib vaccine use would be less in a rural block. The study areas were Khizrabad block (population 168,477) Yamuna Nagar District, Haryana and Sonarpur Block (population 75,000), 24 Pargonas (S) District, West Bengal and Anaicut block (population 128,000), Vellore District, Tamil Nadu. Children aged less than 18 months, with no history of Hib immunization, residing in the study block and willing to use study hospitals were enrolled from the study area from July 2005 to December 2006. All enrolled children were followed until the age of two years or until study stopped on 20th March 2007, whichever came first. At enrollment, demographic, vaccine history and health seeking data was collected by community volunteers for each enrollee. Additionally, families were informed of signs and symptoms of pneumonia and meningitis and hospital locations. Community Volunteers augmented immunization and encouraged participation in the study through

fortnightly visits. Volunteers did not refer children themselves based on symptoms but provided assistance in seeking healthcare at study centers when parents requested the same for a sick child. To facilitate use of the study hospitals and to ensure that access to healthcare was available to study participants, travel fare, cost of hospitalizations, treatment, antibiotics, laboratory investigations and cost of X ray was reimbursed to parents. Upon presentation to a study hospital with suspected pneumonia or meningitis, standardized clinical and laboratory procedures were followed, which are described in detail below. At 24 months of age, or the end of the study (whichever occurred first), community volunteers collected follow up data for each enrollee on illness events during the study, treatments received, and vaccination history. Verbal autopsies were performed for children who died during the study period. The community volunteers and their field supervisors were appointed and trained for data collection from the study area. Standard operating procedures (SOPs) were established for validation of cohort enrollment by supervisors and completeness of cohort enrollment by medical officers on regular basis.

Sample size for the cohort population was estimated on the basis that severe pneumonia incidence would be approximately 3% and suspected meningitis incidence would be approximately 0.3%. Hence, total enrollment of approximately 10,500 children in the cohort study from all the three study sites was considered to be sufficient to provide 80% power to estimate the incidence of severe pneumonia with a confidence interval of +/- 0.3% and the incidence of meningitis with a confidence interval of approximately +/- 0.1%.

Hospital based surveillance study

A systematic sample of children with suspected pneumonia and meningitis were enrolled at study hospitals to evaluate the feasibility of conducting study procedures and to collect clinical specimen for evaluation of diagnostic tests from 1st October 2005 to 20th March 2007. Infants in

the cohort study were included if they presented to one of the study hospitals. This includes those children with signs of pneumonia and meningitis from the cohort populations. There were 16 study hospitals (14 private and 2 governments) in Yamunanagar, 3 in Vellore and 5 in Kolkata.

Case definitions were developed to match ones used in previous studies and by the WHO. Severe pneumonia was defined as cough or difficult breathing or tachypnea and at least one of: lower chest wall indrawing, nasal flaring, grunting, central cyanosis, inability to feed, lethary, unconciousness, or head nodding. Radiological pneumonia was defined as severe pneumonia with radiographic evidence of consolidation meeting the WHO clinical trialist group primary endpoint criteria. *(20)*. Suspected meningitis was defined as history of fever with one of the following signs: stiff neck, bulging fontanelle, history of seizures, poor feeding, lethargy, or change in mental status. Purulent meningitis was defined as CSF with at least one of the following: turbid appearance, white blood cell count $\geq$ 100/mm3 or WBC $\geq$10 with one of either protein >100 mg%, glucose <40mg%, neutrophils $\geq$80%. Lab confirmed Hib meningitis included Hib isolated by culture, latex agglutination test (LAT) or polymerase chain reaction (PCR).

Hospitalized study subjects were managed by regular clinical staff in study hospitals. For a case of suspected pneumonia, chest X- ray was done and X rays from all the three study sites were read by an external expert. Lumber puncture was performed and CSF was collected for cases of suspected meningitis. CSF was plated and gram stained. Culture was performed on both chocolate and sheep blood agar medium with X and V factors. Cell count was performed using an autoanalyser. Biochemistry included cell count, neutrophils, protein, and glucose. LAT was performed on all samples using commercially available kits (Wellcogen kits). PCR testing was

performed on those samples that were culture and LAT negative for Hib. Specimen collection was similar at all the sites and labs were monitored for quality purposes.

Carriage Study

Nasopharyngeal swabs were collected using calcium alginate from children less than 2 years of age who presented for routine immunization at the selected immunization clinics (mix of urban and rural) from August 2005 to January 2007. A sample size of 300 children was calculated to estimate the proportion of children carrying Hib with a precision of +/- 2.5% at carriage rate of 5%. Children with history of Hib vaccination or illness or malformations of the oropharynx were excluded. Children were enrolled in such a manner that an equal number of children are enrolled every month over a 12 month study period could be enrolled with roughly equal numbers in the following age groups: 1-3 months, 4-6 months and 7-23 months. Swabs were sent to institutional labs at all sites in skim milk triptone-glucose-glycerine (STGG) carrier medium and later cultured for common bacterial colonizers, particularly *H. influenzae* and *S. pneumoniae.*

Data collection instruments and analysis

Data collection instruments for cohort, hospital, and the carriage study were identical at all sites. Data collection instruments were first scanned into computer at all the sites and later sent to Society for Applied Studies (SAS), Delhi for data compilation and central analysis. Incidence estimates and 95% confidence intervals were derived using a Poisson regression model adjusted with a Pearson Chi-2 statistic. Data was analyzed using STATA 9 and SPSS version 15.

Case Definitions:

Hospitalization

Any child admitted to the hospital casualty department or inpatient ward for ≥24 hours or any child who dies in any hospital department.

Suspected meningitis (indication for initiating meningitis data collection, including lumbar puncture, in an enrolled child)

1. Hospitalization, and
2. History of fever according to parent report, and
3. One of the following:
   - stiff neck (The child holds the neck back stiffly and resists moving it.)
   - bulging fontanelle (The 'fontanelle' which is the soft spot towards the front of an infants head is 'bulging' i.e. it is pushed out and tense when the infant is in a sitting position and not crying)
   - history of seizures
   - poor feeding, lethargy, or mental status changes

Purulent meningitis

1. Suspected meningitis, and
2. The CSF shows at least one of the following:
   - Turbid appearance
   - WBC $\geq$100/mm^3
   - WBC $\geq$ 10/mm^3 with one of the following:
     · protein >100 mg%
     · glucose <40mg%
     · WBC differential showing $\geq$ 80% neutrophils

Laboratory confirmed Hib meningitis

1. Suspected meningitis, and
2. At least one of the following:
   - Blood culture grows Hib and criteria for purulent meningitis are met or CSF is not available for testing
   - CSF culture grows Hib
   - Hib antigen is detected in CSF
   - Hib DNA is detected in CSF by PCR

Severe clinical pneumonia (indication for initiating pneumonia data collection, including chest X-ray, in an enrolled child)

For this study, we will use the WHO/IMCI definitions for severe pneumonia or very severe pneumonia as shown below.

1. Hospitalization, and
2. Cough or difficulty breathing or tachypnea (respiratory rate $\geq$ 50 for ages 2-11 months and $\geq$ 40 for ages 12-13 months), and
3. At least one of the following:
   - Lower chest wall indrawing
   - Nasal flaring
   - Grunting
   - Central cyanosis
   - Inability to feed
   - Lethargy or unconsciousness

- Head nodding (movement of the head in synchrony with respiration indicating use of accessory muscles)

Radiographic pneumonia

1. Severe clinical pneumonia, and

Radiographic evidence of consolidation (meeting the WHO Clinical Trialists Group primary endpoint criteria)

Primary pneumonia endpoint

1. Severe clinical pneumonia, and
2. At least one of the following:
   - Hib antigen detected in urine
   - Elevated C reactive protein (CRP)
   - Evidence of primary consolidation on chest X-ray as determined by a single reader trained using the WHO Clinical Trialists guidelines.

Invasive Hib disease

1. Hospitalization, and
2. At least one of the following:
   - Identification of Hib in the culture of a normally sterile body fluid (i.e., blood, CSF, pleural fluid, joint fluid)
   - Identification of Hib antigen in a normally sterile body fluid (excluding urine)

Procedures for the evaluation of subjects with suspected pneumonia

Procedure	Test done	Standard of Care	Standard of Care Tests	Research Tests
Chest X-ray	Yes	Yes	Reading	
Venipuncture	Yes	Yes	Blood culture Cell count	C-reactive protein
Urine collection	Yes	Yes		Antigen detection
Nasopharyngeal swab	Yes	No		Culture

Procedures for the evaluation of subjects with suspected meningitis

Procedure	Test done	Standard of Care	Standard of Care Tests	Research Tests
Lumbar puncture	Yes	Yes	Cell count Chemistry Antigen detection Culture	PCR
Venipuncture	Yes	Yes	Blood culture Cell count	C-reactive protein
Urine collection	Yes	Yes		Urine antigen detection
Nasopharyngeal swab	Yes	No		Culture

Results:

Part A Final Report -- Tables

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Table 1: Socio demographic profile of cohort in Chandigarh, Vellore and Kolkata sites

Parameters	Chandigarh N (%)	Kolkata N (%)	Vellore N (%)
Male sex	5147 (54.47)	1600 (50.46)	2560 (50.55)
Median enrollment age (months)	3.55	4.52	4.77
Ever breastfed	9256 (97.95)	3115 (98.23)	4995 (98.64)
Children >=20 weeks with 3 DPT doses	8561 (92.4)	2635 (87.2)	4293 (97.0)
Median (IQR) age of DPT-3 (weeks)	21.1 (17.6-26.7)	21.0 (17.7-26.6)	17.3 (15.7-19.9)
Any Hib doses (at follow-up)	9 (0.1)	17 (0.53)	96 (1.88)
Maternal literacy			
Illiterate	3657 (38.70)	479 (15.11)	223 (4.40)
Can sign or read only	534 (6.02)	308 (9.71)	1265 (24.98)
Can read and write	5259 (55.65)	2384 (75.18)	3576 (70.62)
House where child lives			
No house	31 (0.33)	5 (0.16)	14 (0.28)
Hut or katcha* house	2881 (30.48)	1635 (51.56)	2584 (51.03)
Mixed‡ house	3262 (34.52)	849 (26.77)	836 (16.51)
Pucca£ house or mansion	3276 (34.66)	682 (21.5)	1630 (32.18)

* House which is made of mud with thatched roof

‡House where walls are made of brick but floor is mud. Thatch or cement ceiling.

£House is cement and/or brick

Table2: Child years o f observation across site stratified by age categories:

site	less than six months	six to twelve months	more than 12 months	Total
Chandigarh	**1785.26**	**2300.90**	**4233.09**	**8319.23**
Kolkata	**513.62**	**773.19**	**1591.93**	**2878.74**
Vellore	**778.15**	**1139.98**	**2308.42**	**4226.62**
Total	**3077.04**	**4214.07**	**8133.42**	**15424.59**

Table 3: Hospital admissions among cohort children by site

Site	Number
Chandigarh	**258**
Kolkata	**250**
Vellore	**222**
Total	**730**

Table 4: Discharge diagnosis of pneumonia across sites stratified by age categories:

Discharge diagnosis - pneumonia	One to five months	six to twelve months	more than 12 months	Total
Chandigarh (n)	135	70	41	**246**
Incidence (per 100,000 cyo)	7563 (6309 - 9066)	3034 (2355 - 3907)	967 (683- 1370)	2945 (2525 - 3435)
Kolkatta (n)	82	70	84	**236**
incidence (per 100,000 cyo)	15886 (12869 - 19610)	8650 (7049 - 10614)	5086 (4116 - 6284)	8029 (7063 - 9127)
Vellore (n)	70	40	48	**158**
incidence (per 100,000 cyo)	9068 (7122 - 11545)	3500 (2362 -5186)	1946 (1432 - 2644)	3714 (3030 - 4551)
Total (n)	**287**	**180**	**173**	**640**

Table 5: Discharge diagnosis of pneumonia across sites stratified by age categories:

Discharge diagnosis - pneumonia	One to five months	six to twelve months	more than 12 months	Total
Chandigarh (n)	128	59	40	**227**
Incidence per 100,000 cyo	7168 (5963-8617)	2557 (1972-3316)	943 (676-1315)	**2717 (2313-3191)**
Kolkatta (n)	83	70	78	**231**
incidence per 100,000 cyo	16082 (13 038-19836)	8779 (7166-10755)	4772 (3817-5966)	**7890 (6917-9000)**
Vellore (n)	60	33	38	**131**
incidence per 100,000 cyo	7773 (5998-10 072)	2887 (1840-4530)	1556 (1098-2206)	**3075 (2476-3819)**
Total (n)	**287**	**180**	**173**	**640**

Table 6a: Readable x-rays with primary consolidation at Chandigarh, Kolkata and Vellore sites.

Site	Total X-rays (N)	Readable X-rays (N)	N (%) of readable x-rays with primary consolidation			
			1-5 mo	6-11 mo	12-23 mo	Total
Chandigarh	223	156	8 (10.0)	5 (10.6)	3 (11.1)	16 (10.4)
Kolkata	224	190	1 (1.6)	6 (10.5)	2 (2.9)	9 (4.8)
Vellore	98	88	13 (40.6)	2 (8.0)	9 (29.0)	24 (27.3)

Table 6b: Readable x-rays with any consolidation

	N (%) of readable x-rays with any consolidation			
Site	1-5 mo	6-11 mo	12-23 mo	Total
Chandigarh	12 (15)	7 (14.3)	6 (22.2)	25 (15.5)
Kolkatta	4 (6.25)	12 (21.1)	13 (18.8)	29 (15.3)
Vellore	16 (50.0)	10 (40.0)	14 (45.2)	40 (45.5)

Table 7a: Discharge diagnosis of meningitis

Site	*Discharge diagnosis of meningitis (N)* 1-5 mo	6-11 mo	12-23 mo	Total
Chandigarh	6	5	5	**16**
Kolkatta	0	0	0	**0**
Vellore	5	4	1	**10**

Table 7b: Incidence of discharge diagnosis of meningitis

Site	Incidence of discharge diagnosis of meningitis (95% CI) per 100,000 child years of observation 1-5 mo	6-11 mo	12-23 mo	Total
Chandigarh	339 (138 - 830)	217 (100 - 472)	118 (41 - 340)	192 (101 - 367)
Kolkatta	0	0	0	0
Vellore	648 (301 - 1393)	350 (120 - 1022)	43 (7 - 256)	237 (117-476)

Table 8a: Frequency (N) of suspected clinical meningitis

Site	Frequency (N) of suspected clinical meningitis 1-5 mo	6-11 mo	12-23 mo	Total
Chandigarh	87	46	31	164
Kolkatta	23	20	29	72
Vellore	33	21	35	89

Table 8b: Incidence of suspected clinical meningitis (per 100,000 child years of observation).

Site	Incidence of suspected clinical meningitis per 100,000 child years by age (95% CI)			
	1-5 mo	6-11 mo	12-23 mo	Total
Chandigarh	4910 (3967-6078)	1994 (1463-2716)	731 (484-1105)	**1971 (1617-2404)**
Kolkata	4511 (3045-6681)	2582 (1766-3774)	1695 (1146-2507)	**2433 (1891-3131)**
Vellore	4145 (2926-5872)	1575 (956-2596)	1556 (1103-2196)	**2034 (1594-2595)**

Table 9: Lumbar puncture rate

Site	LP rate per 100,000 child years of observation			
	1-5 mo	6-11 mo	12-23 mo	Total
Chandigarh	336	217	118	192
Kolkatta	0	129	63	69
Vellore	2442	614	477	875

Table 10: CSF correlates of physician diagnosed meningitis cases in hospital based surveillance study in Chandigarh, Kolkata and Vellore sites.

CSF correlates among all LPs	Chandigarh n=90 (%)	Kolkata n=98(%)	Vellore n=181(%)
WBC count ≥100	7 (7.78)	29 (29.6)	38 (21.0)
WBC count 10-99 with protein >100 mg% or glucose <40mg% or neutrophils ≥ 80%	10 (11.0)	11 (11.0)	17 (9.39)
Hib positive on culture/ antigen/PCR	2 (2.22)	7 (7.14)	15 (8.29)
WBC count>100 with Hib positive culture/ antigen	1 (16.7)	6 (20.7)	11 (29.0)

Table 11: Case fatality ratios at discharge

Disease syndrome	Chandigarh %	Kolkata %	Vellore %
Diagnosis of pneumonia	1.01	2.35	0.77
Severe clinical pneumonia	1.35	3.32	0.89
Diagnosis of meningitis	4.71	2.70	2.68
Suspected meningitis	1.54	5.10	0.98
Purulent meningitis	0	24.4	0

Table 12: Carriage rate of haemophilus influenzae type b (Hib) and streptococcus pneumoniae in nasopharynx of study children in Chandigarh, Kolkata and Vellore sites.

Results of nasopharyngeal swab culture	Chandigarh N = 513 (%)	Calcutta N=299 (%)	Vellore N =302 (%)
Haemophilus influenzae type b	39 (7.6)	19 (6.4)	18 (6.0)
Streptococcus pneumoniae	140 (27)	91 (30)	85 (28)

Results

This study demonstrates the incidence of clinical pneumonia and meningitis among Indian children less than 2 years of age in selected rural districts. The incidence of clinical disease described here is similar to what is observed elsewhere prior to the introduction of Hib or Pneumococcal conjugate vaccines. The sample size did not allow for an estimation of the incidence of Hib-specific disease. However, analysis of laboratory samples demonstrated that Hib was present in 16.7-29.0% of CSF samples with greater than 100 WBCs. Findings from this study support the need for universal Hib immunisation in India to prevent serious mortality and morbidity.

Dissemination and Application

Findings from this study have been submitted for publication to the *Indian Journal of Medical Research* (New Delhi), and have been presented by junior investigators at the 2008 International Conference of Infectious Diseases in Kuala Lumpur. Additionally, findings were included in presentations to the National Technical Advisory Group on Immunisation, and thus informed the Government of India's decision to introduce Hib vaccine.