The Seroepidemiology of *Haemophilus influenzae* Type B Prior to Introduction of an Immunization Programme in Kathmandu, Nepal

Andrew S. J. Marshall1*, Charlotte I. S. Barker1, Anoop S. Pulickal1, Elizabeth Kibwana1, Samir C. Gautam2, Elizabeth A. Clutterbuck1, Stephen M. Thorson2, Shrijana Shrestha2, Neelam Adhikari2, Andrew J. Pollard1, Dominic F. Kelly1

1 Oxford Vaccine Group, Oxford University, and the NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom, 2 Patan Hospital, Kathmandu, Nepal

Abstract

*Haemophilus influenzae* type b (Hib) is now recognized as an important pathogen in Asia. To evaluate disease susceptibility, and as a marker of Hib transmission before routine immunization was introduced in Kathmandu, 71 participants aged 7 months–77 years were recruited and 15 cord blood samples were collected for analysis of anti-polyribosylribitol phosphate antibody levels by enzyme-linked immunosorbent assay. Only 20% of children under 5 years old had levels considered protective (>0.15 µg/ml), rising to 83% of 15–54 year-olds. Prior to introduction of Hib vaccine in Kathmandu, the majority of young children were susceptible to disease.

Introduction

*Haemophilus influenzae* type b (Hib) remains a significant cause of invasive bacterial disease globally, and is of particular importance in resource-poor countries. It is estimated to cause approximately 371,000 deaths each year and over 8 million cases of serious disease in children under 5, including pneumonia, meningitis and epiglottitis [1]. The Hib vaccine comprises the Hib surface polysaccharide antigen polyribosylribitol phosphate (PRP) conjugated to a protein carrier. Since 2006, the World Health Organization (WHO) has recommended that this vaccine be included in all routine infant immunization programmes [2]. The WHO advised that “lack of local surveillance data should not delay the introduction of these vaccines”, especially in countries with evidence of a high burden of disease. Despite this, uncertainty has surrounded the relative importance of Hib disease in South East Asia, and adoption of the vaccine has subsequently been slow [3]. With support from the Global Alliance for Vaccines and Immunization, Hib vaccine was introduced in Nepal in 2009 [4].

In unvaccinated populations, repeated exposure to Hib antigens during childhood is thought to lead to natural immunity, with development of protective levels of anti-PRP antibody over time [5]. The aim of this seroepidemiological study, carried out in the pre-Hib-vaccination era in Nepal, was to determine the level of Hib-specific serum antibodies in a sample of the Kathmandu population, in order to evaluate disease susceptibility, and as a surrogate marker of Hib transmission prior to vaccine introduction. In addition these data may provide a useful baseline against which to compare post-vaccination seroepidemiological studies, for example when determining whether a booster vaccine dose will be needed in this population [6].

Materials and Methods

Ethics statement

As part of the original study [7], ethical approval was given by the appropriate Institutional Review Boards (The Nepal Health Research Council and the Oxford Tropical Research Ethics Committee, reference 017-05) for sample storage and use in future studies of vaccine-related immunity. Written consent was provided by all participants or the parents/guardians for those under the age of 18 years.

Participants and samples

The samples were collected as part of a seroepidemiological study described previously [7]. Briefly, patients attending the outpatient department at Patan Hospital, Kathmandu, Nepal, for non-infectious conditions in June and July 2006 were invited to participate. Hib vaccine was available in some private clinics in Nepal at the time, but in practice unavailable to the vast majority of patients attending Patan Hospital. Following fully informed consent, 71 participants aged 7 months–77 years were recruited and 15 cord blood samples were collected.
consent from the patient (or guardian if the patient was <18 years old) venous blood samples were taken. Cord blood samples were obtained from consecutive deliveries in the Patan Maternity Ward where consent was provided. Volunteers with pyrexia, or any immune disorder were excluded. Sera were separated from clotted whole-blood samples by centrifugation and frozen.

Hib ELISA

Serum anti-PRP antibody concentrations were determined using a standard protocol [8,9]. Duplicate sample sera, initially diluted 1:20, were re-tested at 1:200 and/or 1:2000 if necessary.

Statistical analysis

The mean concentration for each sample duplicate was log_{10}-transformed for calculation of the standard error of the geometric mean concentration (GMC) [10]. Any sample below the lower limit of detection (0.1 µg/ml) was given a value of 0.08 µg/ml for the purpose of analysis. Samples were categorized as cord blood or by age of participant in completed years. There is limited evidence regarding the concentration of anti-PRP antibody required for protection from invasive disease [11,12]. As in previous studies, we identified in 25% of cases (n = 77) of bacterial meningitis (with positive cultures) that Hib was the second most common cause of meningitis in children after *Streptococcus pneumoniae* [25,26]. Hib was identified in 25% of cases (n=77) of bacterial meningitis (with established aetiology) in children under 5 [26], which is low

give an indication of the proportion of individuals susceptible to Hib infection whilst the rate of acquisition of anti-PRP antibody between age cohorts should reflect the degree of exposure to Hib or cross-reactive antigens. In our study, 90% of children under 5 were serologically susceptible to invasive Hib disease with concentrations of anti-PRP antibody below the threshold of protection (<0.15 µg/ml). Even in late childhood, a substantial proportion of individuals still did not have protective concentrations of anti-PRP antibody (Fig. 1).

Anti-PRP Acquisition

Comparisons of antibody levels between different Hib seroepidemiological studies are limited by significant inter-laboratory variability in the anti-PRP assay, and even between different labs using the same anti-PRP ELISA [16]. It is therefore difficult to use age-specific serology data to infer disease incidence. However, the profile of anti-Hib antibody acquisition throughout childhood can be compared and clearly differs between countries (see Supporting Information, Figure S1 & Table S1, in File S1). Some studies find that a low proportion of young children have protective concentrations (>0.15 µg/ml) but a rapid increase in the proportion of older children who are protected. For example, in Burkina Faso whilst only 9% of children under 5 years of age had concentrations >0.15 µg/ml, 75% of those aged 4–14 years were protected [17]. This suggests a relatively high degree of exposure to Hib in the under 5 s, and correlates with Hib meningitis incidence data from Burkina Faso [18], and other African countries, which suggest a high proportion of cases in infants, and a high incidence in the under 5 s. Carriage data from West Africa also support a high exposure to Hib in this region [19]. Seroepidemiological studies from some other regions, including the UK [20], display a more gradual increase in the proportion protected with increasing age. Overall, however, it is difficult to identify a clear relationship between the different population serologic profiles (Figure S1 & Table S1, in File S1) and either the estimated incidence of Hib meningitis under 5, or the proportion of Hib meningitis cases in infants [18]. Contributing factors that may obscure a relationship between anti-PRP antibody and Hib exposure are the variability in anti-PRP assays and the levels of exposure to cross-reactive antigens [21]. Of all studies identified, our data demonstrated one of the slowest rates of acquisition of protection (<0.15 µg/ml), from 20% under 5 years of age, to 40% in those aged 5–7 years, 67% in 8–14 year-olds, and 83% at 15–54 years. This suggests a relatively low level of Hib exposure compared with that observed in other countries in the pre-vaccine era.

Carriage

In populations with a known burden of invasive Hib disease, reported carriage rates vary from 5 to 9% in children under 5 years of age [22]. Such data for Nepal are sparse, but Williams *et al.* reported a Hib carriage prevalence of 5% in children aged 3 months to 12 years in the pre-vaccination era [4]. This is comparable to countries such as the USA and UK [23,24], where disease incidence has been sufficient to warrant routine immunization.

Disease incidence

Although there have been no population incidence data for invasive Hib disease reported from Nepal, two studies prior to the introduction of routine Hib immunisation from Kathmandu demonstrated that Hib was the second most common cause of meningitis in children after *Streptococcus pneumoniae* [25,26]. Hib was identified in 25% of cases (n=77) of bacterial meningitis (with established aetiology) in children under 5 [26], which is low...
compared with most studies globally, as meta-analysed by WHO [18], but still indicative of a significant burden of disease. By inference, Hib pneumonia is likely to be prevalent but difficult to demonstrate as cases are rarely bacteraemic [25,26]. Five South-East Asian studies in the WHO analysis [18], and one of the studies from Kathmandu [26], demonstrated a high proportion of total Hib meningitis cases occurring in infancy. Although it has been suggested that the proportion of infant cases is positively related to overall disease incidence, the correlation is relatively weak, making inferences about incidence unreliable for any individual country.

Limitations
The limitations of our study include the small sample size which precludes a more detailed age-specific analysis of Hib immunity acquisition in childhood, for example in those under 2 years who would be expected to have impaired anti-PRP responses to natural Hib exposure. There were only 5 children under 2, none of whom had protective levels of antibody (>0.15 μg/ml). Also, the small blood volumes in young children were insufficient to allow comparisons of antibody avidity. Avidity has previously been proposed as a biomarker for successful induction of immunological memory, with increased avidity representing more functional antibody [9]. Measuring antibody avidity may identify an effective immunological memory (secondary to ‘natural priming’) in subjects with low antibody concentrations [27]. Selection bias may have been introduced by the hospital-based nature of this study, the patient charges at Patan Hospital (although local medical staff consider that the costs are not sufficient to deter attendance) [25], and the attributes of patients willing to participate in a research study of this kind. Finally the waning anti-PRP levels measured in the elderly may reflect comorbidities, which were not solicited in this study.

Implications
The importance of Hib in Asia has previously been questioned. However, our data suggest that at least 83% of the Kathmandu population had been naturally exposed to Hib, or cross-reactive antigens, before adulthood. Although our seroepidemiological data are most compatible with a lower level of exposure to Hib carriage, previous data on Hib carriage prevalence [4], and meningitis aetiology [25,26] still indicate the importance of Hib disease in Nepali children. Furthermore, we identified a relatively prolonged period for which children are at risk of a disease for which carriage and aetiological studies support a significant burden of invasive illness, with the majority of children under 8 being susceptible to Hib. Now that a Hib conjugate vaccine is included in the national immunization schedule, we would expect Hib transmission to be low, through reduced acquisition amongst vaccinees. Since this will in turn reduce the opportunity for natural boosting of Hib antibody levels, ongoing serological surveillance could be used to inform the need for booster doses of vaccine, using data in this study as a baseline.

Supporting Information
File S1 Table S1 and Figure S1. Comparison of Hib seroprevalence studies in the pre-vaccine era. (DOC)
Acknowledgments

We thank the participants in the study, including parents of the children, and the staff of Patan Hospital.

References

1. Watt JP, Wollson LJ, O’Brien KL, Henkle E, Deloria-Knoll M, et al. (2009) Burden of disease caused by Haemophilus influenzae type b in children younger than 5 years: global estimates. Lancet 374: 903–911.

2. WHO (2006) Position paper on Haemophilus influenzae type b conjugate vaccines. (Replaces WHO position paper on Hib vaccines previously published in the Weekly Epidemiological Record, Wkly Epidemiol Rec 81: 445–452

3. Ojo LR, O’Loughlin RE, Cohen AL, Loo JD, Edmond KM, et al. (2010) Global use of Haemophilus influenzae type b conjugate vaccine. Vaccine 28: 7117–7122.

4. Williams EJ, Lewis J, John T, Hoe JC, Yu L, et al. (2011) Haemophilus influenzae type b carriage and novel bacterial population structure among children in urban Kathmandu, Nepal. Journal of clinical microbiology 49: 1323–1330.

5. Oh SY, Griffiths D, John T, Lee YC, Yu LM, et al. (2008) School-aged children: a reservoir for continued circulation of Haemophilus influenzae type b in the United Kingdom. J Infect Dis 197: 1275–1281.

6. Metz JA, Hanish E, Pradhan R, Joshi A, Shakyta D, et al. (2012) Evaluation of haemophilus influenzae type b vaccine for routine immunization in Nepali infants. Pediatr Infect Dis J 31: e66–72.

7. Pulickal AS, Gautam S, Clutterbuck EA, Thorson S, Basynat B, et al. (2009) Kinetics of the natural, humoral immune response to Salmannella enterica serovar Typhi in Kathmandu, Nepal. Clinical and vaccine immunology: CVI 16: 1413–1419.

8. Booy R, Taylor SA, Dobson SR, Isaacs D, Sleight G, et al. (1992) Immunogenicity and safety of PRP-T conjugate vaccine given according to the British accelerated immunisation schedule. Archives of disease in childhood 67: 475–478.

9. Kelly DF, Moxon ER, Yu LM, Pollard AJ (2009) Anti-polyribosylribitol phosphate antibody concentrations and avidities in children since the start of Haemophilus influenzae type b immunization of infants in the United Kingdom. Clinical and vaccine immunology: CVI 16: 246–252.

10. Heath PT, Booy R, Azopardi HJ, Slack MP, Bowen-Morris J, et al. (2000) Antibody concentration and clinical protection after Hib conjugate vaccination in the United Kingdom. JAMA 284: 2334–2340.

11. Kayhyn H (1994) Difficulties in establishing a serological correlate of protection after immunization with Haemophilus influenzae conjugate vaccines. Biologicals journal of the International Association of Biological Standardization 22: 397–402.

12. Kelly DF, Moxon ER, Pollard AJ (2004) Haemophilus influenzae type b conjugate vaccines. Immunology 113: 163–174.

13. Kayhyn H, Peltola H, Karanko V, Makela PH (1983) The protective level of serum antibodies to the capsular polysaccharide of Haemophilus influenzae type b. The Journal of infectious diseases 147: 1100.

14. Makela PH, Peltola H, Kayhyn H, Jou¥mines H, Pettay O, et al. (1977) Polysaccharide vaccines of group A Neisseria meningitidis and Haemophilus influenzae type b: a field trial in Finland. The Journal of infectious diseases 136 Suppl: S43–50.

15. Forthergill LD, Wright J (1935) Influenza Meningitis - The Relation of Age Incidence to the Bactericidal Power of Blood Against the Causal Organism. J Immunol 24: 273–284.

16. Madore DV, Anderson P, Baxter BD, Carlone GM, Edwards KM, et al. (1996) Interlaboratory study evaluating quantitation of antibodies to Haemophilus influenzae type b polysaccharide by enzyme-linked immunosorbent assay. Clinical and diagnostic laboratory immunology 3: 84–89.

17. Ballereau F, Speich M, Apaire-Marchais V (1999) Natural Haemophilus influenzae type b capsular polysaccharide antibodies in 412 infants and children from West Africa (Burkina-Faso) and France: a cross-sectional serosurvey. Eur J Epidemiol. 15: 577–582.

18. Bennet J P, Slack M, Peter Mala A (2002) Haemophilus influenzae type b (Hib) meningitis in the pre-vaccine era: a global review of incidence, age distributions, and case-fatality rates. Vaccines and Biologicals, WHO, 2002.

19. Adegbola RA, Secka O, Lahai G, Lloyd-Evans N, Njie A, et al. (2005) Elimination of Haemophilus influenzae type b (Hib) disease from The Gambia after the introduction of routine immunization with a Hib conjugate vaccine: a prospective study. Lancet 366: 144–150.

20. Trotter CL, McMinnon J, Andrews NJ, Burrage M, Ramsay ME (2003) Antibody to Haemophilus influenzae type b after routine and catch-up vaccination. Lancet 361: 1523–1524.

21. Leino T, Auranen K, Makela PH, Kayhyn H, Ramsay M, et al. (2002) Haemophilus influenzae type b and cross-reactive antigens in natural Hib infection dynamics: modelling in two populations. Epidemiology and infection 129: 73–83.

22. Olsen SJ, Dejardin S, Sanguk L, Chumritwat S, Dowell SF (2003) Frequent Haemophilus influenzae type b colonization in rural Thailand. Pediatr Infect Dis J 24: 739–740.

23. Barbou ML, Mayon-White RT, Coles C, Crook DW, Moxon ER (1995) The impact of conjugate vaccine on carriage of Haemophilus influenzae type b. The Journal of infectious diseases 171: 93–98.

24. Michaels RH, Poziviak CS, Stonebreaker FE, Norden CW (1976) Factors affecting pharyngeal Haemophilus influenzae type b colonization rates in children. Journal of clinical microbiology 4: 413–417.

25. Kelly DF, Thorsen S, Maskey M, Mahat S, Shrestha U, et al. (2011) The burden of vaccine-preventable invasive bacterial infections and pneumonia in children admitted to hospital in urban Nepal. International journal of infectious diseases 15: e17–23.

26. Shah AS, Knoll MD, Sharma PR, Moisi JC, Kulkarni P, et al. (2009) Invasive pneumococcal disease in Kanti Children’s Hospital, Nepal, as observed by the South Asian Pneumococcal Alliance network. Clin Infect Dis 48 Suppl 2: S123–126.

27. Goldblatt D, Vaz AR, Miller E (1998) Antibody avidity as a surrogate marker of successful priming by Haemophilus influenzae type b conjugate vaccines following infant immunization. The Journal of infectious diseases 177: 1112–1115.

Author Contributions

Conceived and designed the experiments: AM AJP DK. Performed the experiments: AM ASP SG ST SS NA. Analyzed the data: AM CB AJP DK. Contributed reagents/materials/analysis tools: AM ASP EG EC AJP DK. Wrote the paper: AM CB AJP DK. Optimised the ELISA: AM EG. Designed the original study [reference 7], obtained ethical approval and recruited participants: ASP SG ST SS NA.