***Haemophilus influenzae* type b (Hib) seroprevalence and current epidemiology in England and Wales**

Sarah Collins,1 David Litt,2 Rachael Almond,3 Jamie Findlow,3 Ezra Linley,3 Mary Ramsay,1 Ray Borrow,3 Shamez Ladhani.1,4

1 Immunisation Department, Public Health England, London, UK

2 Respiratory and Vaccine Preventable Bacterial Reference Unit, Public Health England, London, UK

3 Meningococcal Reference Unit, Public Health England, Manchester, UK

4 Paediatric Infectious Diseases Research Group, St. George’s University of London, London, UK

**Corresponding Author:** Dr Shamez Ladhani, Immunisation Department, Public Health England, 61 Colindale Avenue, London NW9 5EQ, UK. E-mail: [shamez.ladhani@phe.gov.uk](mailto:shamez.ladhani@phe.gov.uk)

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**Abstract:**

**Introduction**

The introduction of the Hib conjugate vaccine in the United Kingdom resulted in a rapid decline in invasive Hib disease across all age groups. However, a resurgence in 2000-2002 prompted the introduction of additional control measures, including a routine 12-month booster in 2006. Here we describe results from a national serosurvey in children eligible for the 12-month booster and recent *H. influenzae* epidemiology in England and Wales.

**Methods**

A national serosurvey was performed to determine the prevalence of anti-polyribosyl-phosphate (anti-PRP) IgG antibodies in 1,000 residual samples from children up to 8 years of age in 2013-14. Data were compared to previous national serosurveys performed by the same laboratory. Current epidemiology of invasive *H. influenzae* disease in England and Wales is also reported.

**Results**

Median anti-PRP IgG concentrations were highest among 1 year olds at 4.5μg/mL (95%CI, 0.93-33.6; n=55) and then declined rapidly but remained ≥1.0 µg/mL across the age-groups in the cohort eligible for the 12-month booster. Overall, 89% of children (719/817) had anti-PRP concentrations ≥0.15 μg/mL, the putative threshold for short-term protection against invasive Hib disease. During 2012-2016, annual Hib disease incidence remained below one case per million population, with only 67 of 3,523 (2.5%) laboratory-confirmed *H. influenzae* cases and one case of Hib meningitis during the 5-year period. There were only two deaths within 30 days over the five-year period (case fatality rate, 3.0%).

**Conclusions**

Hib control in England and Wales is currently the best achieved since the vaccine was introduced more than two decades ago. However, Hib antibodies wane rapidly after the 12 months booster. Although most children remain protected against disease, antibody levels may not be high enough to prevent carriage among toddlers. Ongoing monitoring is essential to inform future vaccination policy.

**Keywords** *Haemophilus influenzae* type b, clinical presentation,immunisation, risk factors, seroprevalence, outcome

**INTRODUCTION**

*Haemophilus influenzae* can be distinguished into six serotypes (Hia-f), defined by their capsular polysaccharide, or can be unencapsulated (nontypeable *H. influenzae*, ntHi). Prior to routine immunisation, serotype b (Hib) was a major cause of bacterial meningitis in young children and was associated with significant morbidity and mortality worldwide [1]. The introduction of the Hib conjugate vaccines into national childhood immunisation programmes more than two decades ago led to a rapid and sustained reduction in the incidence of invasive Hib disease across all age groups through direct and indirect (herd) protection [2].

In England, the Hib conjugate vaccine was introduced into the routine childhood immunisation programme in 1992 as a three dose infant priming schedule at 2, 3, and 4 months alongside a catch-up campaign for all children up to four years of age [3]. During 2000-02, an increase in Hib cases, initially among toddlers but soon followed across all age groups, led to a number of control measures, including changing to a more immunogenic vaccine (DTwP-Hib) in 2002, a toddler booster campaign in 2003 offering a dose of the Hib conjugate vaccine to all children who were only immunised in infancy, routine 12-month Hib/MenC combination booster in 2006, and a limited pre-school booster campaign for children who were too young for the 2003 booster campaign and too old for the 12 month booster [4]. These measures resulted in a rapid and sustained decline in the incidence of invasive Hib disease across all age groups, and the disease has remained well-controlled since then [5].

Following recent reports of rapid waning of immunity and effectiveness of the meningococcal C (MenC) vaccine in toddlers after the 12-month booster [6], we conducted a national seroprevalence study to measure Hib antibodies in children in 2015. Here, we also describe the current epidemiology of invasive *H. influenzae* disease in England and Wales, as well as the clinical characteristics and outcomes of invasive Hib disease during the past five years.

**METHODS**

***National Seroprevalence***

Participating NHS laboratories routinely submit residual sera from routine diagnostic testing to the Sero-Epidemiology Unit [7]. In total, 1,000 sera from children up to 8 years of age during 2013-2014 were selected.  Children in this age group would have been eligible for the 12-month booster, in addition to the three infant priming doses at 2-3-4 months, which was implemented in 2006.

Antibody concentrations (IgG) against the Hib capsular polysaccharide (polyribosyl-ribitol phosphate (PRP)) were quantified using a fluorescent bead assay, as described previously [9]. Briefly, PRP was conjugated to carboxylated microspheres (Luminex Corporation; Texas, United States) following bead activation (via a two-step carbodiimide reaction). Serum was diluted 1:100 and a standard curve prepared using the World Health Organization (WHO) international standard TE-3. Diluted preparations were added to a filter plate (Millipore, Watford, UK) and mixed with conjugated beads. Following incubation, the plate was washed and anti-human IgG-RPhycoerythrin (RPE) added to each well. Following incubation and washing, the plate was read on a BioPlex workstation (BioRad, Hertfordshire, UK) and analysis undertaken using Bioplex manager software, with a four parameter logistic fit model.

Antibody concentrations (IgG) against the Hib capsular polysaccharide (polyribosyl-ribitol phosphate, PRP) were quantified using a fluorescent bead assay. Thresholds were defined as an anti-PRP IgG concentration of ≥0.15μg/mL for short-term protection against invasive Hib disease, ≥1μg/ml for long-term protection. These thresholds are based on previous animal experiments, studies on natural immunity, passive immunisation and the original clinical trials of Hib-PRP polysaccharide vaccines, which suggested that minimum anti-PRP IgG concentrations of 0.05–0.15 μg/ml at the time of exposure to the organism were required to protect against invasive disease [8,9].

Seroprevalence results were compared to three previous seroprevalence studies which used the same source of samples and laboratory testing methodology: (i) 1993–94, when the Hib conjugate vaccine had just been introduced into the national childhood immunisation programme; and (ii) 1995–2001, when routine infant Hib conjugate vaccination was in place; and (iii) 2009, following the various childhood boosters and the routine 12-month dose introduced in 2006. Median values with interquartile ranges (IQR) are reported because anti-PRP IgG concentrations were highly skewed and not normally distributed even when log-transformed. The Mann-Whitney U test was used to compare medians. Categorical variables are expressed as proportions and compared using the chi-squared test.

***National Surveillance***

Public Health England (PHE) conducts enhanced national surveillance of IPD in England and Wales. Hospital laboratories routinely report clinically significant infections electronically to PHE and submit invasive *H. influenzae* isolates to the PHE reference laboratory for serotyping. For reported cases without isolate submission, PHE actively contacts the reporting laboratory to submit the isolate to the PHE reference laboratory for confirmation and serotyping. Invasive *H. influenzae* disease was defined as isolation of the organism from a normally sterile site. Bacterial isolates were confirmed as *H. influenzae* by their growth requirement for X and V factors [10], and *ompP2*-specific polymerase chain reaction (PCR) positivity [11]. Capsulation status was determined by PCR using bexA-specific primers [12]. Capsular type was confirmed by capsule-specific PCR using primers for types Hia–f [13] and slide agglutination using monovalent Hia–f antisera [10]. Laboratory-confirmed invasive Hib disease diagnosed during 2009-2015 were followed up using a standardised questionnaire to the patient's general practitioner. Annual population estimates were obtained from the Office for National Statistics. Poisson 95% confidence intervals were calculated for incidence rates.

***Ethical Approval***

National Research Ethics Service (NRES) approval for the sero-epidemiological surveillance of the National Immunisation programme of England and Wales (Research Ethics Committee number 05/Q0505/45) was granted by the Joint University College London/ University College London Hospital (UCL/UCLH) Committees on the Ethics of Human Research. PHE has legal permission, provided by Regulation 3 of The Health Service (Control of Patient Information) Regulations 2002, to process patient confidential information for national surveillance of communicable diseases (<http://www.legislation.gov.uk/uksi/2002/1438/regulation/3/made>). This includes PHE’s responsibility to monitor the safety and effectiveness of vaccines.

**RESULTS**

***Seroprevalence***

Of the 1,000 residual samples, based on the date of sample and age in months of the child, 805 belonged to children in the age group eligible for the 12-month booster that introduced in September 2006. The median anti-PRP IgG concentrations were highest among 1 year olds at 4.4 μg/ml (IQR, 1.3-14.9; n=99) and then declined rapidly to 2.1 μg/ml (IQR, 0.6-5.6; n=98) in 2 year-olds and 1.2 μg/ml (IQR, 0.40-3.2; n=100) in 3 year-olds (**Figure 1**). Median Hib concentrations remained ≥1 g/mL across the age-groups in the cohort eligible for three infant priming doses followed by the 12-month booster.

Overall, 89% of children (719/817) had anti-PRP concentrations ≥0.15μg/mL, the putative threshold for short-term protection, while 56% (448/807) had anti-PRP concentrations ≥1.00 μg/mL, the putative threshold for long-term protection against invasive Hib disease (**Figure 2**). However, only 23% (183/624) %) had anti-PRP concentrations ≥5.00 μg/mL, the putative threshold for protection against Hib carriage; proportions increased from 22% in 6-11 month-olds to peak at 45% among 1 year-olds and then declining to 27% in 2 year-olds, 15-22% among 3-7 year-olds and 25% among 8 year-olds.

***Epidemiology***

During 2012-2016, the incidence of invasive Hib disease has remained very low across all age-groups (< 0.05 cases per 100,000 population), with a total of 67 laboratory-confirmed cases reported to PHE during this 5-year period (**Figure 3**). The incidence of invasive disease due to other serotypes has remained stable (0.14-0.20 cases per 100,000 population), while ntHi incidence has been increasing gradually from 0.12 cases/100,000 in 1990 to 1.2 cases/100,000 in 2016.

During the 5-year surveillance period, there were 3,523 confirmed cases in England and Wales, including 2,883 (81.8%) that were serotyped. Of these, only 67 (2.3% were identified as Hib, 365 (12.7%) were other serotypes (4 Hia, 2 Hid, 85 Hie and 272 Hif) and 2,451 (85.0%) were ntHi.

Of the 67 Hib cases, 11 (16%) were confirmed in children, including 5 in infants (aged <1 year), four in toddlers (1-4 year-olds), two in older children (5-14 year-olds). The remaining 56 cases (84%) were diagnosed in adults, including 15 in 15-44 year-olds, 26 in 45-64 year-olds and 14 in ≥65 year-olds. Of the cases with serotyped isolates, Hib was responsible for 2.0% of cases in <1 year-olds, 2.9% in 1-4 year-olds, 3.2% in 5-14 year-olds, 3.9% in 15-44 year-olds, 5,4% in 45-64 year-olds and 0.9% of 65+ year-olds (Table). Of the 5 infant cases – all with positive blood cultures – one was too young to be immunised and the other four had received one (n=1), two (n=1) or three (n=2) doses before developing invasive disease at 8-11 months of age. Of the four toddler cases, three were fully immunised with 4 doses and one was unimmunised. Hib was isolated from the CSF in one case and the blood in the remaining cases

Only 2.0% (72/3,523) of the isolates were from the cerebrospinal fluid (CSF); of these 72 isolates, 54 were serotyped isolates and only one was identified as Hib. Other serotypes were responsible for 8 of the 54 cases with serotyped isolates: two (1 Hie, 1 Hif) of six cases in infants, four (all Hif) of nine cases in toddlers and two (1 Hie, 1 Hif) of 12 cases in 65+ year-olds (Table). The remaining were all identified as ntHi. Over the 5-year period, there were only two deaths within 30 days (case fatality rate, 3.0%; 95% CI, 0.4%-10.4%), both among 45-65 year-olds with underlying comorbidities.

**DISCUSSION**

The UK is currently experiencing a period of excellent Hib control across all age groups, 25 years after the vaccine was first introduced into the national childhood immunisation programme and 10 years after the introduction of a routine 12-month booster. This has been achieved through consistently high vaccine coverage in the childhood immunisation programmes, reaching 93.5% for the 3 infant priming doses by 12 months and 91.6% for the 12-month booster cove.Public [14] Currently, the incidence of invasive Hib disease is less than 1 case per million population, with nearly all cases occurring in adults.

We undertook a seroprevalence study to assess the immune status of children who would have been eligible for the 12 month Hib booster which was introduced in September 2006. This additional dose was added to the national immunisation programme after the national surveillance identified poor long-term protection in toddlers following three infant priming doses without a booster in the second year of life [15].

In September 2006, the UK also modified the infant meningococcal C conjugate vaccine (MCC) schedule from 3-dose infant priming schedule only to a 2-dose priming schedule at 2-3 months followed by a booster at 12-13 months. By 2010, however, MCC vaccine effectiveness was observed to decline rapidly from 96% in the first 12 months after vaccination, to 68% after 2 years, 60% after 3 years and only 31% thereafter [16]. Cases of MenC disease, however, remained low, especially in children, most likely because of the population protection offered by the initial catch-campaign targeting all individuals up to 25 years of age in 1999. In June 2013, however, one of the infant MCC doses was moved to the adolescent age group to maintain long-term population control because older teenagers are the main carriers of *Neisseria meningitidis* and conjugate vaccines prevent acquisition of carriage and onward transmission to others; in August 2015, this adolescent dose was replaced with the meningococcal ACWY conjugate vaccine to combat a national outbreak of group W meningococcal disease due to a hypervirulent strain belonging to the ST-11 clonal complex [17].

In the current seroprevalence study, we also observed rapid waning of Hib antibody concentrations after 1 year of age, with anti-PRP concentrations stabilising just above 1 µg/mL from three years of age. We have previously shown rapid waning of antibody in healthy toddlers who had been immunised against Hib and MenC in infance followed by the 12-month Hib/MenC booster [18]. The higher antibody anti-PRP concentrations at 8 years of age in the current cohort is likely to be due to a small proportion of 8 year-olds who would have received the Hib conjugate vaccine with their pre-school booster as part of the 2007-2009 Hib booster campaign [4]. This limited campaign targeted children who would have been too old for the 12-13 month booster but too young for the 2003 Hib booster campaign and is responsible for the large peak in Hib antibody concentrations among toddlers in the 2009 serosurvey (Figure 1).

it is, however, reassuring to note that nearly all the children eligible for the 12-month Hib booster had anti-PRP antibody concentrations above the putative threshold for short-term protection and more than half had antibody concentrations conferring long-term protection. Serum antibodies play a critical role in protection because of the rapid disease progression following invasion by encapsulated bacteria such as Hib [19]. Our results confirm that most children are adequately protected with the current Hib immunisation programme and this is evidenced by the low national incidence.

In September 2013, the UK introduced an emergency national antenatal immunisation programme against pertussis using a combination vaccination vaccine that included the diphtheria and tetanus antigens.[20] At the time, there were concerns that these additional antigens might interfere with immune responses to conjugate vaccines in the infant immunisation programme that utilised tetanus-toxoid and the diphtheria toxin-related CRM197 proteins as their protein conjugates. We subsequently showed higher immunogenicity for tetanus-associated antigens, including tetanus-toxoid, as well as the Hib and MenC conjugate (which are both conjugated to tetanus toxoid) in infants whose mothers were vaccinated in pregnancy compared to those born to unimmunised mothers [21]. Vaccine responses to diphtheria toxoid and CRM-conjugated antigens were less predictable, with evidence of immune interferences for some of the antigens. Therefore, children immunised after the seroprevalence was undertaken may be better protected against Hib because of the antenatal immunisation programme.

For the prevention of carriage, it has been proposed that higher antibody concentrations up to 5 µg/mL may be required [22,23]. Carriage studies after the introduction of the Hib conjugate vaccines have consistently reported zero or low carriage rates, especially in toddlers who were previously the main carriers of Hib [24]. That cases continue to occur sporadically across all age groups suggests that there may still be on-going circulation, highlighting the importance of maintaining high vaccination rates for those who are the most at risk, especially given that most adults are currently susceptible to Hib because of lack of natural boosting [7]. We are unable to confirm whether the recent Hib cases are associated with travel as has been reported for adult MenC cases [16]. In older adults, we have previously reported that most invasive Hib cases occur in those with chronic respiratory conditions who develop lower respiratory tract infections, similar to ntHi infections in this age group [5]. Given the rarity of Hib cases overall and among meningitis cases, even in children, the current UK recommendations for the prevention of secondary cases could be restricted to recommending chemoprophylaxis for close contacts of confirmed Hib cases only (<https://www.gov.uk/government/publications/haemophilus-influenzae-type-b-hib-revised-recommendations-for-the-prevention-of-secondary-cases>).

**STRENGTHS and LIMITATIONS**

The strength of the current study is the national surveillance that has been in place for more than three decades, alongside a national reference laboratory offering a free confirmation and serotyping of invasive isolates. Since serotyping is not routinely performed in local laboratories, this service ensures high case ascertainment with high serotyping rates for all invasive isolates. An important limitation, however, is that only invasive isolates are serotyped and included in the surveillance, although *H. influenzae* is known to be a major cause of upper and lower respiratory tract infections, especially at the extremes of age.[25]

Another major strength of the current analysis is the multiple national seroprevalence studies since the introduction of Hib immunisation programme. As with all seroprevalence studies, however, the findings must be interpreted with caution. The residual sera from participating National Health Service (NHS) hospital laboratories may not be representative of the general population. However, the large number of samples does allow comparison by age and with previous seroprevalence studies, which used sample sources from similar geographical regions and were tested using the same methodology and in the same laboratory.

**CONCLUSIONS**

The incidence of invasive Hib disease remains extremely a decade after the addition of a routine 12-month booster into the national childhood immunisation programme. Although anti-PRP antibody concentrations wane rapidly after the 12-month booster, nearly all children maintain sufficiently high concentrations to confer at least short-term protection against invasive Hib disease. The continued small numbers of sporadic cases in adults highlights the importance of maintaining high vaccine coverage in children who are the main reservoirs of Hib.

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COVER REFERENCE:

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| --- | --- | --- | --- | --- | --- |
|  | Hib | Other  encapsulated | Non-typeable | Not  serotyped | All cases |
| <1y | 5 (0) | 16 (2) | 225 (4) | 31 (2) | 277 (8) |
| 1-4y | 4 (1) | 30 (4) | 106 (4) | 36 (2) | 176 (11) |
| 5-14y | 2 (0) | 10 (0) | 50 (2) | 19 (2) | 81 (4) |
| 15-44y | 16 (0) | 34 (0) | 365 (11) | 95 (2) | 510 (13) |
| 45-64y | 26 (0) | 70 (0) | 389 (12) | 122 (6) | 607 (18) |
| 65+y | 14 (0) | 205 (2) | 1313 (10) | 326 (3) | 1858 (15) |
| Not Reported | 0 (0) | 0 (0) | 3 (2) | 11 (1) | 14 (3) |
| Total | 67 (1) | 365 (8) | 2451 (45) | 640 (18) | 3523 (72) |

**Table.** Laboratory-confirmed cases of invasive *Haemophilus influenzae* disease and meningitis (numbers in parenthesis) by serotype in England and Wales over a five-year period between 2012 and 2016.

Figure 1.

Comparison of median *Haemophilus influenzae* serotype b (Hib) anti-polyribosyl-ribitol phosphate (PRP) IgG antibody concentrations by age in years in the 2013-14 seroprevalence study with three previous seroprevalence studies conducted across England and Wales using the same methodology and in the same laboratory

**Figure 2.** Comparison of proportion of children with *Haemophilus influenzae* serotype b (Hib) anti-polyribosyl-ribitol phosphate (PRP) IgG antibody concentrations (A) ≥ 0.15 g/mL and (B) ≥ 1.0 g/mL by age in years in the 2013-14 seroprevalence study with three previous seroprevalence studies conducted across England and Wales using the same methodology and in the same laboratory. Hib antibody concentrations ≥ 0.15 g/mL and ≥ 1.0 g/mL refer to the putative levels considered as respectively providing short-term and long-term protection against invasive Hib disease.

**Figure 3**. Incidence (with 95% confidence intervals) of laboratory-confirmed invasive *Haemophilus influenzae* disease in England and Wales during 1990-2016. Hib = *Haemophilus influenzae* serotype b; Non-b = encapsulated *Haemophilus influenzae* other than Hib; NTHi = non-typeable *Haemophilus influenzae*