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Do we need a booster of Hib vaccine after primary vaccination? A study on anti-Hib seroprevalence in Sweden 5 and 15 years after the introduction of universal Hib vaccination related to notifications of invasive disease

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The prevalence of IgG ELISA antibodies against *Haemophilus influenzae* polyribosyl ribitol phosphate (anti-Hib) was studied in two Swedish seroepidemiologic materials. One study was performed in 1997 5 years after the introduction of universal Hib vaccination (N = 3320). Ten years later, a similar study was carried out to analyze the effect of vaccination on anti-Hib prevalence (N = 2383). The median values of anti-Hib concentrations (EU/mL) were almost identical in the two materials. The antigenic pressure including vaccination, natural infections and possible cross-immunizations was thus assumed to be constant. The joint median was 0.50 EU/mL (95% confidence interval: 0.46, 0.56). However, there were also indications of reduced exposure to 'Hib-antigens' over a 10-year period. The proportion above the cut-off point for protection, 0.15 EU/mL, decreased significantly for children aged 2–19 years from 78% in 1997 to 74% in 2007 (p = 0.034), and there was a significant increase in values below the minimal level of detection for adults from 17% in 1997 to 20% in 2007 (p = 0.009). In the 2007 material no specific age group could be identified with a lower immune profile than other age groups older than 3 years and there was a significant downward trend of invasive infections caused by Hib according to notification data for the period 1997–2008. Therefore, the conclusion is that presently there is no need for a booster dose of Hib vaccine in Sweden after primary vaccination but the situation should be carefully monitored.

Key words: *Haemophilus influenzae*; vaccination; ELISA IgG anti-Hib; seroepidemiology; notifications.

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Haemophilus influenzae (Hi) is a primary human pathogen but is also part of the normal nasopharyngeal flora, especially in young children. There is no known non-human reservoir. Invasive disease is in general caused by encapsulated strains, having the capacity to avoid phagocytosis and complement-mediated killing. Based on the capsular polysaccharide, six serotypes have been identified (a–f). Those with a polyribosyl ribitol phosphate (PRP) capsule, named Hib, have been seen most often in serious bacteremic disease manifested as meningitis, bacteremic pneumonia, epiglottitis,

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septicemia, cellulitis and osteoarticular infections (1–3). Meningitis represented 52% of the Hib disease spectrum as reviewed by Peltola from 21 studies carried out during the 1970s until the 1990s. The vast majority of cases affected children younger than 5 years. During the pre-vaccination era, the worldwide yearly incidence of meningitis in children aged 0–4 years was estimated to be 57 per 100 000 (4).

In Sweden, the mean incidence of invasive Hi disease (meningitis and septicemia) in children younger than 5 years of age was 34.4 cases per 100 000 before the vaccination era (5). About 95% of all invasive diseases in children were owing to serotype b. The median age for invasive Hi cases was 2 years 4 months during the period 1987–1991.

Polysaccharide–protein conjugate vaccines that induce protective antibodies to the type b capsule have successfully reduced the disease burden. This is true also in small children (<2 years), who normally have poor capacity to produce antibodies against T-independent carbohydrate antigens.

Children born in Sweden are recommended three doses of conjugated vaccines against Hib since 1993 at 3, 5 and 12 months of age. The coverage rapidly reached approximately 98.5%, a level shown to be more or less constant since their introduction (6). Invasive disease type b is mandatory notifiable since 1996 according to the Communicable Disease Act. In 2004 notification of invasive Hi disease of all types became mandatory.

After vaccination was introduced in Sweden, the incidence of invasive Hi disease decreased rapidly in the youngest age group. In 1994, the second year after the introduction, an incidence of 3.4 per 100 000 was seen among the children aged 0–4 years. The effectiveness of Hib vaccination in the birth cohort of children from 1993 to 1997 in Sweden was calculated to be 96.1%. No significant decline was observed in older age groups (5).

Surveillance data from 1997 to 2003 showed an average incidence of 0.3 per 100 000 of invasive Hib disease (children 0–4 years; 1.1) with a median age of 49.5 years (7).

Occasional cases of vaccine failure have been reported each year globally. In the United Kingdom, an increased incidence of vaccine failures between 2000 and 2002 was associated with reduced immune response to Hib because of the use of a Hib vaccine preparation that was combined with a diphtheria, tetanus, three-componenet acellular pertussis vaccine (DTPa3-Hib) and the absence of an early booster dose in the vaccination schedule (8–10).

Anti-capsular, opsonizing antibodies, following the natural history of infection, ensure protection against Hib. With age, there is an increase in anti-PRP antibodies inversely related to the incidence of invasive Hib disease (3). Concentrations of anti-PRP ≥ 0.15 μg/mL have been considered adequate to provide short-term protection against invasive disease (11–14). When ELISA techniques are used, the results of measurement are often expressed as EU/mL directly traceable to and translated from the primary FDA reference serum (μg/mL). A problem with vaccination may be that conjugate vaccines not only prevent disease but also consequently reduce the normal Hib carriage with loss of natural boosters and herd immunity (10, 15).

Population-based immunity studies offer a tool to find out whether the prevalence of anti-Hib antibodies has changed in different age groups after the introduction of vaccination. In Sweden, two such materials were available and analyzed, one collected in 1997 and one in 2007. A follow-up of notified cases was also performed to find out whether the observed levels of concentration were consistent with an effect on the incidence of disease.

MATERIALS AND METHODS

Vaccines

When vaccination was first introduced, the Act-HIB vaccine, Sanofi Pasteur MSD, was used. After 1997 this vaccine has been replaced by the combination vaccines Infanrix-Polio + Hib (GlaxoSmithKline, Rixensart Belgium) and Pentavac (Sanofi Pasteur MSD, Lyon France).

Seroepidemiologic blood samples from studies during 1997 and 2007

In 1997 and 2007 cross-sectional seroepidemiologic surveys were performed in Sweden. In both surveys, children as well as adults from the Swedish population were selected in randomized samples, stratified by age as described in a previous study (16).

In 1997 altogether 6000 individuals were randomly selected from the population register in 10 age groups. Blood samples were analyzed for ELISA immunoglobulin G (IgG) anti-Hib antibodies from 3120 of planned 3600 individuals sampled in this way (17).

In addition, cord blood was taken from 200 newborn children, consecutively at three maternity hospitals and analyzed for ELISA IgG anti-Hib antibodies. Thus, altogether 3320 blood samples were analyzed for IgG anti-Hib antibodies in the 1997 seroepidemiologic survey.

In 2007, 2383 sera samples were analyzed for ELISA IgG anti-Hib antibodies from planned 4600 individuals. Of analyzed samples, 377 were cord blood samples. Cord blood samples (N = 200) were taken consecutively at 10 randomized maternity hospitals. Before performing statistical analysis, the cord blood results were classified according to the age group of mothers.

Comparison of the sero-surveys 1997 and 2007

A summary of included and excluded age groups is presented in Table 1. Age groups in bold in Table 1 were used for comparison of sero-surveys 1997 (N = 3320) and 2007 (N = 1998). Age groups sampled in 2007 were analyzed and new ones were constructed to be as comparable as possible with age groups sampled in the 1997 survey. Altogether 385 individuals were excluded, leaving 1998 for analysis. See also under the 'Statistics' section.

Table 1. Distribution by age in years in Swedish seroepidemiologic materials from 1997 and 2007. Figures in bold represent number of individuals used for comparison of seroprevalence between 1997 and 2007

1997		2007	
Age group	Samples	Age group	Samples
2–2.4	**100**	2	**53**
		3	55
5–5.4	**128**	4–5	**122**
		6–7	95
8.4–9.3	**199**	8–9	**119**
10.4–11.3	**253**	10–11	**132**
		12–13	146
14.4–15.3	**200**	14–15	**153**
		16	62
17.4–18.3	**212**	17–18	**116**
		19	27
20–34	**569**	20–34	**105**
35–49	**463**	35–49	**159**
50–64	**519**	50–64	**260**
65–	**477**	65–	**402**
Cord blood	**200**	Cord blood	**377**
Total	3320	Total	2383

SEROLOGY

IgG to anti-Hib by ELISA

An indirect ELISA was used at the Swedish Institute for Infectious disease Control (SMI) to measure the concentrations of IgG antibodies to Hib-PRP (anti-Hib). The general outlines follow those for anti-diphtheria and anti-pertussis antibodies described in previous studies (18, 19).

Hib polysaccharide conjugated to human serum albumin HbO-HA, lot 17 (Wyeth Pharmaceuticals Inc, Collegeville, PA, USA), diluted to 1 mg/L in phosphate-buffered saline, pH 7.4, was used as antigen for coating high-binding polystyrene plates overnight at 37 °C.

The results were internationally traceable to the FDA (Food and Drug Administration, USA) *Haemophilus influenzae* Type b Reference Serum lot 1983 assigned 60.9 μg/mL of IgG and used as calibrator. A pool of fractioned human plasma (Ig42) containing 46 EU/mL and diluted 1 + 3 was used as a monitor. The minimum level of detection (MLD) was 0.06 EU/mL.

The results are expressed in arbitrary units (EU/mL), directly traceable to and translated from the primary FDA reference serum (μg/mL).

With defined limits for acceptance and adequate retesting, the within-day coefficient of variation was less than 15% and the drift over time was also less than 15%. Before each new study, a panel of 19 sera was tested to ensure traceability.

The method was accredited and the laboratory participated in an external quality assessment program. Protective level was defined as 0.15 EU/mL. Sera from children 15 years and younger collected in 1997 were tested in 2002. All other sera were tested in 2009.

Reporting of Hi disease

Invasive disease with Hib was notifiable between 1996 and 2004 according to the Communicable Disease Act. Other types were reported to the SMI within the voluntary laboratory reporting with an estimated underreporting of 11%. In July 2004 invasive disease also of other types became notifiable. Cases are reported with full personal identifiers both by physicians and laboratories. Data about clinical course are not

required in the reporting form and are therefore not available. To get detailed clinical information about the cases by reviewing patient journals, a separate study with the approval from the ethical committee is needed.

Ethics

The regional ethical review board in Stockholm approved the seroepidemiologic study on 21 April 1997 (Dnr 97-092) and 28 February 2007 (Dnr 2007/132-31/4). Written informed consent was obtained from the participants.

Statistics

As data are heavily skewed and censored by MLD, all tests that have been carried out are non-parametric using no specific assumption on underlying distribution. One-sided Wilcoxon tests have been used to test the decrease in median values between 1997 and 2007 in various sub-groups. A Kruskal–Wallis test was also used to test the equality of median values in age groups older than 3 years in 2007.

Testing differences in data distributions has been performed with two-sample Kolmogorov–Smirnov tests with the maximum difference between reversed cumulative frequency curves as a test statistic. Again, one-sided tests were used to detect a decrease in antibody concentrations in the population. Finally, difference in proportions with respect to age groups and incidences of invasive infection with Hib over time have been tested using the standard normalized one-sided z-test and trends in proportions have been tested using the chi-squared test.

RESULTS

Anti-Hib concentrations in 1997 and 2007

The total anti-Hib concentrations are first presented as reverse cumulative distribution curves with the cut-off points 0.15 and 1 EU/mL inserted as vertical lines Figs. 1 and 2. The lower level is an internationally accepted concentration related to protection. The 1 EU/mL level was originally also used as a marker of protection but will in the present study rather be used as an indicator of natural booster.

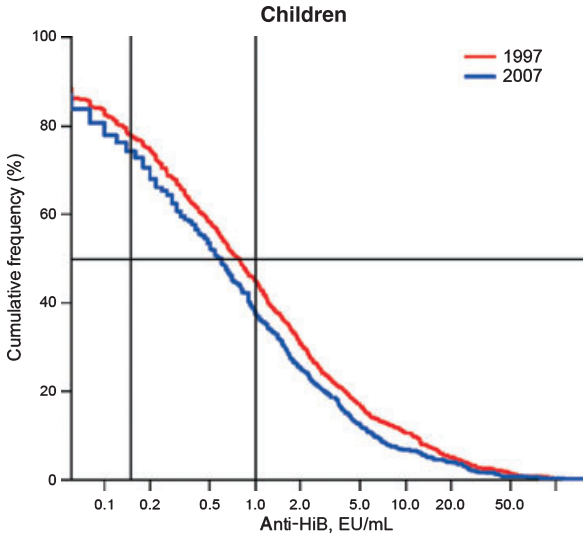

Fig. 1. Sero-survey 1997 and 2007. Reverse cumulative distribution curves of ELISA immunoglobulin G anti-*Haemophilus influenzae* type b antibody concentrations (EU/mL) by comparable age groups in children 1–19 years of age. Vertical lines indicate cumulative prevalence at 0.15 and 1 EU/mL.

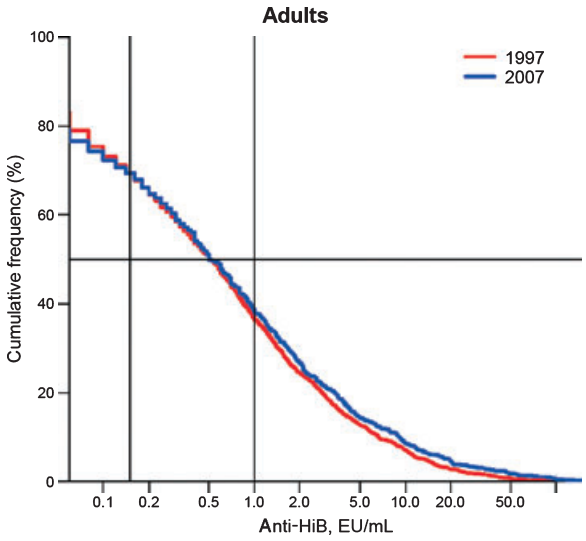

Fig. 2. Sero-survey 1997 and 2007. Reverse cumulative distribution curves of ELISA immunoglobulin G anti-*Haemophilus influenza* type b antibody concentrations (EU/mL) in adults by comparable age groups. Vertical lines indicate cumulative prevalence at 0.15 and 1 EU/mL.

Test of median values – First the median values of selected groups in 1997 and 2007 (Table 1) were compared, for children aged 2–19 years

Table 2. p-values for one-sided tests of decreasing anti-*Haemophilus influenzae* type b for children (2–19 years), adults (20 years and older) and cord blood

	Children	Adults	Cord blood
Test of medians (Wilcoxon)	< **0.001**	0.64	0.23
Test of distributions (Kolmogorov–Smirnov)	< **0.001**	0.16	< **0.001**
Test of proportion < MLD	0.22	**0.009**	0.059
Test of proportion > 0.15 EU/mL	**0.034**	0.48	0.40
Test of proportion > 1 EU/mL	**0.001**	0.79	0.50

MLD, minimum level of detection. Bold values indicate statistical significance.

(Fig. 1), for adults and for cord blood (Fig. 2). With the statistical hypothesis that the antigenic pressure of Hib was lower in 2007 than in 1997, the only statistical significance was found for children as shown in Table 2 ($p < 0.001$).

Looking at median values for specific age groups (Fig. 3), those 14–15 years and 17–18 years of age showed significantly lower median values in 2007 than in 1997 ($p < 0.001$ and $p = 0.020$, respectively).

It can also be noted that the median values in both materials dropped from 1.64 EU/mL at the age of 2 years to approximately 0.6 EU/mL at 4–5 years of age (Fig. 3). For older age groups, the median values continued to be below 1 EU/mL suggesting that values above this level could also be used as an indicator of natural booster.

Test of distributions – These tests show if there is a significant difference at any point of the curve. Significant differences were found for children and cord blood ($p < 0.001$) but not for adults. For cord blood, the median values were 0.35 EU/mL in 1997 and 0.40 EU/mL in 2007, but the 1997 and 2007 curves intersect, indicating that the population in 2007 was more heterogeneous with both more low values and more high ones. This might be a sampling effect, as cord blood was consecutively sampled at a small number of clinics, or an age effect, as the age distribution in 2007 was more spread out with a larger number of older mothers than in 1997.

Trends by age – In 2007, there was no significant difference ($p = 0.18$) between age groups older than 3 years. Hence, the antigenic pressure including vaccination, natural boosters and possible cross-immunization can be assumed to be constant in this group resulting in a joint median of 0.50 EU/mL (95% confidence interval: 0.46, 0.56).

Proportions below MLD

The proportion of individuals with no measurable concentrations of anti-Hib might be an indicator of reduced herd immunity because of

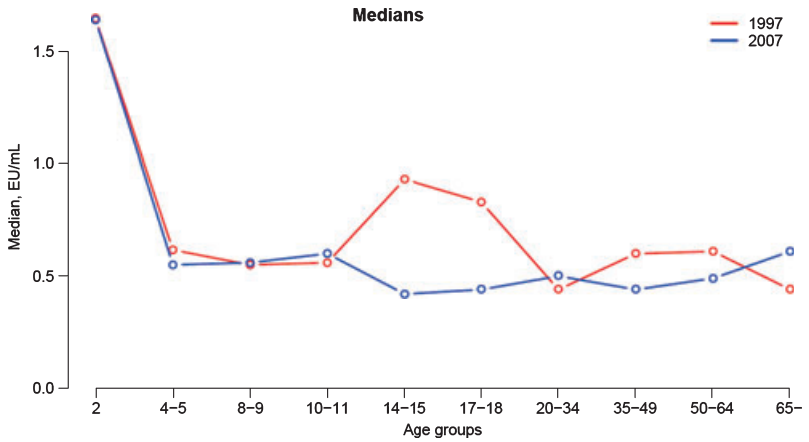

Fig. 3. Sero-survey 1997 and 2007. Anti-*Haemophilus influenzae* type b median concentrations, EU/mL, distributed by age groups.

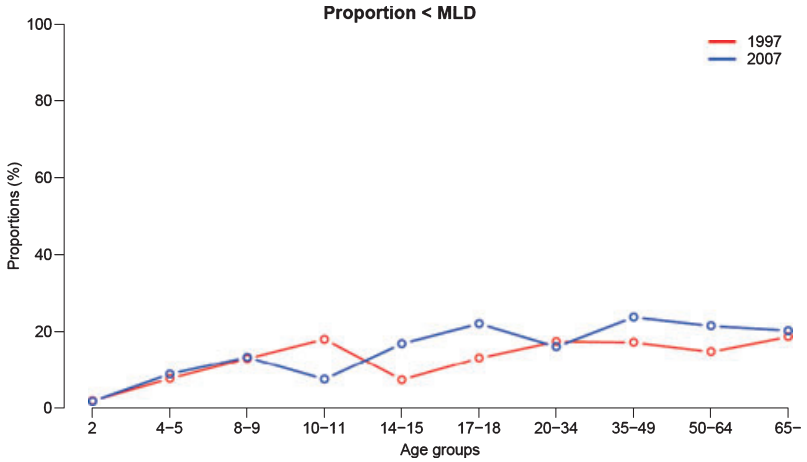

Fig. 4. Sero-survey 1997 and 2007. The proportion of sera with concentrations, EU/mL, below minimum level of detection distributed by age groups.

a decrease in antigenic pressure. In these materials, there was a significant increase in values below the MLD for adults from 17% in 1997 to 20% in 2007 ($p = 0.009$; Table 2 and Fig. 4). For cord blood, the corresponding figures were 14% in 1997 and 20% in 2007 ($p = 0.06$). However, among children, it is worth noticing that there was a higher proportion among those 10–11 years of age. After that, the proportion decreased at the same age as the proportion of samples with concentrations above 1 EU/mL increased possibly as a result of 'recent infection'.

Proportions of those with ≥0.15 EU/mL and ≥1 EU/mL

The proportions above the cut-off point for protection, 0.15 EU/mL (Fig. 5A), decreased significantly ($p = 0.034$) for children from 78% in 1997 to 74% in 2007. In 1997 the oldest vaccinated cohort was about 5 years of age and in 2007 about 15 years of age.

An even larger decrease was observed in proportions above 1 EU/mL (Fig. 5B), where the proportion dropped from 45% in 1997 to 37% in 2007 for children ($p = 0.001$; Table 2). Interestingly, the increase in individuals with concentrations ≥1 EU/mL in 1997 started at the age of 8–9 years with a proportion of 41% and is seen up to 17–18 years where the proportion was 48%. A trend test for monotone trend yielded a p-value of 0.035. In 1997 the 8–9-year cohort was the first one that was unvaccinated. Possibly, the increasing trend in 1997 could be an effect of increasing social activity with age. The absence of a similar trend in 2007 is perhaps because of the fact that the circulation of the pathogen was lower.

In the group of children aged 2 years, 97% were above the protection level (Fig. 5A) in 1997 and 96% in 2007. After that, the proportion decreased to about 70% in older cohorts. A one-sided trend test revealed that there was a weakly significant ($p = 0.045$) downward trend in proportions above 0.15 EU/mL with respect to age for those older than 3 years in 2007. There was no similar trend for proportions above 1 EU/mL ($p = 0.17$).

For cord blood, the proportion of samples above the two levels was practically identical (68% in 1997 and 67% in 2007 for 0.15 EU/mL and 29% in 1997 and 30% in 2007 for 1 EU/mL).

Invasive infections with Hi, 1997–2008

This study reveals trends indicating that the exposure to 'Hib-antigens' among adolescents is reduced over a 10-year period. To see if this trend had any correspondence in the clinical reality, we also studied the number of notified cases in different age groups.

During the 12–year period, that is, 1997–2008, there was an increasing trend of reported cases with invasive Hi of all serotypes affecting all ages according to the combined reporting

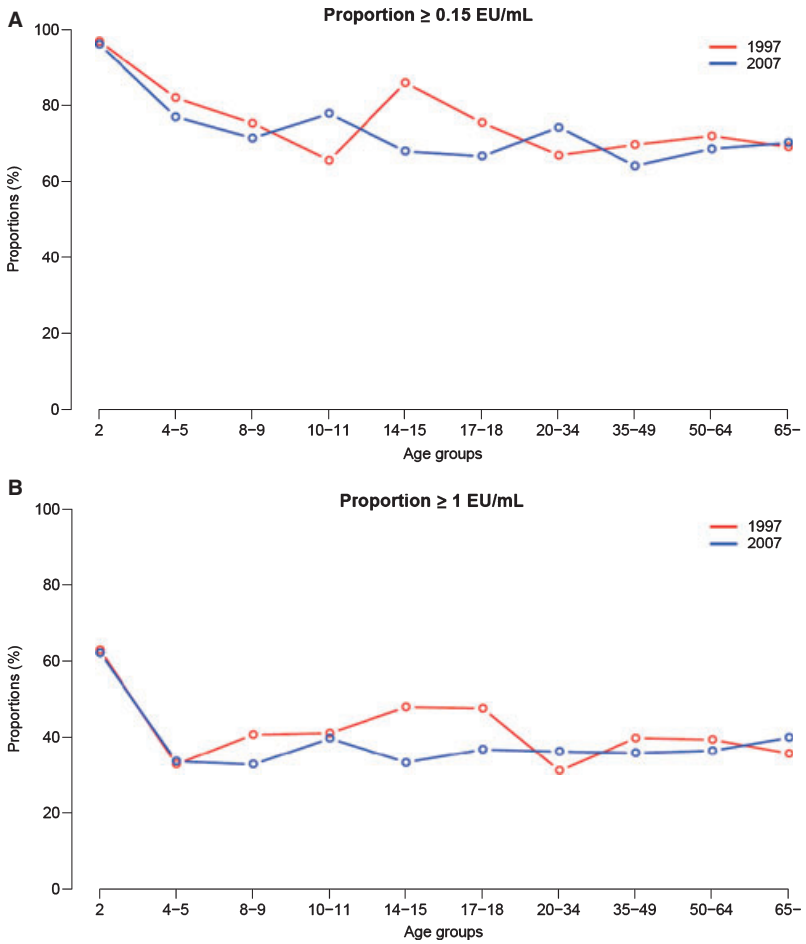

Fig. 5. Sero-survey 1997 and 2007. The proportion of sera with concentrations ≥0.15 EU/mL (A) and ≥1 EU/mL (B) distributed by age groups.

(notifiable and voluntary). Over the same period of time, the number of Hib cases decreased although not to zero (Fig. 6). Altogether, 1475 cases were notified, 302 (20%) of which were of the b type. The incidence of invasive Hib disease was 0.5 per 100 000 in 1997 and 0.16 per 100 000 in 2008. Trend tests showed a significant reduction in invasive Hib incidences during 1997–2008 both for the whole material and for the age group of 0–18 years ($p < 0.001$). Data about serotypes are missing for about 40% of reported cases in 2005–2006, for 13% in 2007 and for 5% in 2008.

In 2005–2008, the average age group-specific incidence rate for invasive Hi infection of all types for children aged 0–4 years was 1.4 per 100 000 (range: 0.8–1.8). Average incidence for

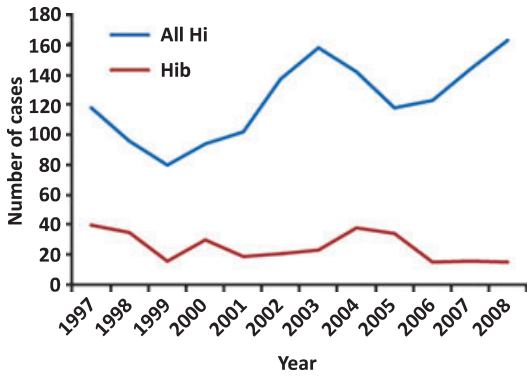

Fig. 6. Number of notified cases of invasive *Haemophilus influenzae* infections in Sweden from 1997 to 2008. The lower line indicates type b whereas the upper line indicates all types. 1996–2003 only Hib was mandatory notifiable. Other types were reported within the voluntary reporting system.

invasive disease type b was 0.4 per 100 000 in the same age group (range: 0–0.8).

During 2001–2008, 17 cases of true vaccine failure were reported in children 5 months to 11 years of age. The total number of Hib cases in children (< 15 years of age) was 31 for the same period.

The median age for all Hi cases has increased from 52 years in 1995 to 69 years in 2008. The median age for Hib cases was 50 years in 1997 and 57 years in 2008 (mean age: 48 resp. 50 years).

DISCUSSION

In Sweden, universal vaccination against Hib was introduced in the childhood immunization program in 1993. It is of general interest to study the effect of this step on the circulation of Hib and the natural re-exposure to maintain herd immunity. For this purpose, sero-surveys play an important role in the evaluation of vaccination programs. The prevalence of anti-Hib concentrations in randomized samples was therefore studied among all age groups in 1997 and in 2007.

A general observation is that the changes in anti-Hib seroprevalence between 1997 and 2007 in total are small. The median concentration of anti-Hib was about 0.50 EU/mL both in 1997 and 2007. The proportion of samples above the internationally accepted antibody level for protection, 0.15 EU/mL, however, was significantly lower for children up to 19 years in 2007, 74% to be compared with 78% in 1997.

There are also signs of less exposure to Hib. Among adults, the proportion of samples with anti-Hib concentrations below MLD was significantly higher in 2007 than in 1997.

Among children, the median values and the proportion of samples > 1 EU/mL were significantly lower in 2007. It is of particular interest that the 1997 and 2007 curves for children started to separate at the age of 8–9 years with a higher proportion above 1 EU/mL in 1997, indicating more infections. In 1997 children up to 4–5 years of age were included in the universal vaccination program that started in 1993. In 2007 also, children aged 14–15 years had been vaccinated. It may be that the more favorable situation in 2007 was because of a prolonged vaccination effect.

In the Netherlands (20), a large-scale population-based sample from 1995 to 1996 showed that the anti-Hib antibodies declined during 2.5 years after the fourth vaccination dose at 11 months of age to 0.8 μg/mL. Among adults, the Geometric Mean Titre (GMT) declined from 1.46 for the age of 20–24 years to 0.73 μg/mL for the age of 75–79 years. Scheifele et al. (21) in Canada studied 4- to 5-year-old healthy children previously given four doses of PRP-T vaccine (at 2, 4, 6 and 18 months) and found a GMT of 2.2 μg/mL and only 0.9% were below 0.15 μg/mL. In the United Kingdom, 153 children at a median age of 3.6 years had a GMT of 1.06 μg/mL. The children had received three doses of PRP-T, DTP and oral polio vaccine by 5 months of age. Eight per cent had an undetectable antibody concentration (8).

Unfortunately, there was no Swedish material available for the analysis of anti-Hib kinetics but it is reasonable to believe that the decay curve for anti-Hib is biphasic with a first rapid part and a second slower one as for anti-pertussis toxin antibodies and diphtheria antitoxin (22, 23). This is supported by the rapid decline of anti-Hib concentrations between 2 and 4/5 years of age and the absence of a significant trend for older age groups.

The maintenance of protective antibody levels is probably because of natural boosting. In the pre-vaccine era, 3–5% of healthy preschool children in developed countries were asymptomatic Hib carriers. Takala et al. found 3.5% oropharyngeal carriers of Hib among unvaccinated 3-year-old healthy children, whereas none of the children who had received Hib conjugate vaccine carried Hib (15). Gambian children were followed up for 4 years after vaccination with Hib/PRP-T DTP. Carriage was significantly lower in this group (4.4%), than in a group vaccinated with DTP alone (11.0%) (24).

Although there is an effect of vaccination on carriage rate, it is evidently not large enough to influence on the population immunity. An option to natural Hib boosting may be cross-immunization with other bacteria such as *Escherichia coli* K1 and K100 (25). Against this explanation speaks the significant difference in anti-Hib levels of the age group 14–15 years in 1997 and 2007.

The incidence of invasive Hib infections has decreased by 95–98% in United States, Canada and many countries in Western Europe after the introduction of conjugated Hib vaccines (4, 5, 26, 27). Hib nowadays accounts for only a small portion of invasive disease cases. Nontypable Hi is the etiologic agent in most of these cases.

Although Hib conjugate vaccines are highly effective, occasional cases of invasive Hib disease in fully vaccinated children are still reported. In the United Kingdom, a resurgence of Hib disease occurred between 1999 and 2003 and was partially attributed to lower immunogenicity of combination vaccines (9). In a group of 855 children aged 6–16 years, prevalence of Hib carriage was estimated to be 4.2% (28).

Failures may be not only due to waning immunity but also to changes in the microbial flora. Schouls et al. (29) found two lineages of Hib strains in the Netherlands. Type 1 strains emerged after the introduction of Hib vaccination and produced twice as much surface-bound capsular polysaccharide as Type II strains. Cerquetti et al. showed that a higher proportion of strains carried multiple copies of the *cap* locus among vaccine failures (30).

The possibility of a negative effect of lower exposure on herd immunity made us also analyze how the notifications of invasive Hi infections and vaccine failures had developed over the years. In Sweden, the incidence of invasive Hib infection has fallen significantly since vaccination was introduced. A few of the cases are true vaccine failures.

In conclusion, the population immunity, expressed as the proportion of individuals with anti-Hib concentrations below the cut-off for protection of 0.15 EU/mL, has been rather stable around 25% over a 10-year period, although a small but significant increase for children up to 19 years of age was observed. In the 2007 material, no specific age group could be identified with a lower immune profile. Moreover, there is a significant downward trend of invasive infections caused by Hib. Therefore, the conclusion is that there is no need for a booster dose of Hib vaccine in Sweden after the primary vaccination. As, however, there are signs of decrease in population immunity over time, particularly among children, the situation should be carefully monitored.

Trial Registration: ClinicalTrials.gov Identifier: NCT 00932269.

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