Antibodies to *Haemophilus influenzae* Serotype b in The Netherlands a Few Years after the Introduction of Routine Vaccination

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We assessed antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b (HibPS) in the Dutch population a few years after a mass vaccination against *H. influenzae* (Hib) was begun. We observed sharp declines in the geometric mean titer (GMT) and the prevalence of HibPS antibodies at levels of \(<0.15 \mu g/mL\) in children who had received 4 doses of vaccine: from 8.65 \(\mu g/mL\) (prevalence, 99.4%) after 0–2 months to 0.8 \(\mu g/mL\) (prevalence, 83.3%) after 27–29 months. In adult groups, both the prevalence of HibPS antibodies and the GMT declined significantly with increasing age but remained high (prevalence, \(\geq 83.7\%\); GMT, 0.73 \(\geq \mu g/mL\)). We conclude that the overall immunity in the Dutch population seems satisfactory. We draw our conclusions from the current serosurveillance study and from the sharp decline in invasive Hib disease noted after the introduction of vaccination. The key questions for the future are (1) whether Hib and cross-reacting organisms will circulate sufficiently to provide natural reexposure, and (2) how long memory immunity will persist after vaccination without reexposure.

Before the introduction of routine vaccination, *Haemophilus influenzae* bacteria with capsular serotype b (Hib) were traditionally the most important cause of invasive *H. influenzae* infections (95%) in The Netherlands. In \(\approx 50\%\) of cases, Hib infections manifested as meningitis (predominantly in infants aged 6–12 months), and in 15%–30% of cases, they manifested as epiglottitis (predominantly in children aged 2–3 years) [1, 2]. Invasive Hib infections cause cellulitis, arthritis, pericarditis, and osteomyelitis at lower incidences.

In The Netherlands, all children born on or after 1 April 1993 are offered free vaccinations against Hib for the prevention of invasive Hib disease. The tetanus toxoid conjugated polyribosylribitol phosphate (PRP-T) vaccine is administered simultaneously with the diphtheria-tetanus-pertussis/inactivated poliomyelitis virus (DTP-IPV) vaccine, but it is injected into another limb. This used to be done at 3, 4, 5, and 11 months of age, but on 1 January 1999, the timing was changed to 2, 3, 4, and 11 months of age. Parents can request vaccinations for children born before 1 April 1993, but they must pay for these vaccinations.

The primary series of Hib vaccination provides 96% coverage. The number of Hib isolates sent to the national reference laboratory has decreased by \(\approx 95\%\) since the introduction of vaccination [1]. When Hib vaccination coverage is high and, as a consequence, invasive Hib infections become rare, efficacy studies are no longer feasible, and serological predictors become critical markers of vaccine efficacy [3]. Although natural
immunity to Hib undoubtedly involves immunity to several surface antigens of the organism, antibodies to the capsular polysaccharide of Hib (HibPS) appear to be of primary importance; they protect against invasive disease caused by this organism [4, 5].

We assessed HibPS antibodies in the Dutch population in late 1995 and in 1996—that is, a few years after mass vaccination against Hib was begun. In this manner, we obtained insight regarding naturally acquired, age-specific antibody levels and the first effects of vaccination on HibPS antibody levels. Furthermore, antibody levels were linked to vaccination status and to questionnaire data on known risk factors for Hib disease, such as day-care attendance and crowding [6, 7].

PATIENTS AND METHODS

A cross-sectional, population-based serosurveillance study was performed in The Netherlands from October 1995 through December 1996. The study design is described in detail elsewhere [8]. In brief, samples from 8 municipalities were drawn in proportion to their populations in each of 5 geographic regions of approximately equal population size. Within each municipality, an age-stratified sample (age strata were 0 years, 1–4 years, 5–9 years, etc., to 75–79 years) of 380 persons was drawn. The first 2 age strata were oversampled because of an expected lower response in these groups, as was observed in a pilot study [9].

Eligible individuals were asked to complete a questionnaire, to give a blood sample at a special clinic, and to show us their vaccination certificates from the national vaccination program. Serum samples of sufficient volume for testing for HibPS antibodies were available from 7864 of the 8359 participants. Most participants (95%) for whom we did not have sufficient serum sample for testing were ≤2 years of age. Their available serum samples were used for testing for antibodies to other diseases that could be prevented by administration of vaccines.

Storage and laboratory tests. The collected serum samples were stored at −86°C. Serum samples were analyzed by use of an ELISA, according to methods previously described elsewhere [10, 11]. HibPS antibody levels (µg/mL) were calculated with a reference serum sample (assigned value, 70 µg/mL total antibodies to HibPS; obtained from Carl Frash, Center for Biological Evaluation and Research, Food and Drug Administration, Bethesda, MD). The 4-parameter fit of this curve was used to interpolate the serum sample absorbance in the linear part of the curve. Antibody levels were categorized into 3 groups: <0.15 µg/mL, 0.15–0.99 µg/mL, and ≥1.0 µg/mL. It has been suggested that an antibody concentration of at least 0.1–0.15 µg/mL at the time of the assay provides protection [12–14], whereas a concentration of 1.0 µg/mL has been regarded as a predictor of long-term protection after vaccination with unconjugated HibPS vaccines [15]. Because the limit of the sensitivity of the ELISA was 0.10 µg/mL, all values <0.10 µg/mL were assigned a value that was 50% of the minimum (0.05 µg/mL) for the calculation of the geometric mean titer (GMT) through log transformation.

Data analyses. The overall frequencies and (geometric) means within a municipality were estimated by weighting the proportion of the age group in the municipality [16]. Because the population sizes of the regions were similar, and because the municipalities were drawn proportionally to their sizes, frequencies and means were averaged over the 40 municipalities to obtain nationwide estimates.

Trends in both the GMT and seroprevalence with increasing age were statistically tested with use of the Pearson correlation test and the χ² linear trend test, respectively. Differences between groups, with regard to the GMT and seroprevalence, were tested using the Wilcoxon rank-sum test. A P value of <.05 was considered statistically significant. Data on age, sex, marital status, nationality, urbanization, region, and reminder by telephone or mail were available for all participants and nonparticipants. The effect of differential probabilities of nonresponse for these variables on both sample estimates was <1 standard error and therefore was ignored [17].

Vaccine. Only 1 Hib vaccine and 1 DTP-IPV vaccine are licensed in The Netherlands. The PRP-T vaccine (10 µg of PRP) is manufactured by Aventis Pasteur MSD, and the DTP-IPV vaccine is manufactured by the National Institute of Public Health and the Environment (RIVM) in Bilthoven, The Netherlands.

RESULTS

Unvaccinated cohorts. In figure 1, both the age-specific prevalence of HibPS antibodies at levels ≥0.15 µg/mL and ≥1.0 µg/mL and the GMT are shown. The prevalence of antibodies and the GMT gradually declined with increasing age in the adult groups; the prevalence of antibodies at levels ≥0.15 µg/mL decreased significantly, from 94.0% in individuals aged 20–24 years to 83.7% in individuals aged 75–79 years (χ² trend, 23.3; P < .0001), and the GMT decreased from 1.46 µg/mL to 0.73 µg/mL (Pearson correlation coefficient, −0.95; P = .0001). Among children aged 3–7 years, the prevalence of antibodies and the GMT were both lower than they were among the children who were younger or older.

Cohorts eligible for vaccination. Longitudinal interpretation of the data for participants who were eligible for vaccination (those born after 1 April 1993) showed that the prevalence of antibodies to HibPS at levels ≥1.0 µg/mL and ≥0.15 µg/mL (the following percentages in parentheses refer to the latter level) increased from 14% (43%) at 2 months to 80% (92%) at 6 months. The prevalence then decreased to 59%
(89%) at 11 months and increased to 89% (96%) in the group aged 12–17 months (figure 1). Thereafter, another decrease was visible: prevalence was 69% (100%) for the group aged 24–29 months, 73% (98%) for the group aged 30–35 months, and 55% (82%) for the group aged 36–43 months (not shown in figure 1).

The GMT showed a similar pattern. It increased from 0.1 μg/mL at 2 months to 4.1 μg/mL at 6 months; decreased to 1.4 μg/mL at 11 months; increased again to 7.6 at 12 months; and then decreased to 2.1 μg/mL for the group aged 24–29 months, decreased to 1.7 μg/mL for the group aged 30–35 months, and decreased to 0.9 μg/mL for the group aged 36–43 months.

To obtain insight into the prevalence and GMT of HibPS antibodies among children receiving vaccinations according to the schedule, we restricted the analysis to those children who were born either on or after 1 April 1993 and who had 4 vaccinations registered, with the fourth vaccination having been given at 10–14 months of age (n = 308; figure 2). The prevalence of antibodies at levels ≥1.0 μg/mL and ≥0.15 μg/mL (percentages in parentheses refer to the latter level) was 96.4% (99.4%) at 0–2 months after the fourth vaccination to 2.1 μg/mL at 6–8 months; thereafter, it slowly declined to 0.80 μg/mL at 27–29 months after the fourth vaccination.

Sociodemographic predictors of HibPS antibodies. The age-specific and overall prevalences of HibPS antibodies and the GMT were consistently slightly higher for female participants than for male participants, but this was not statistically significant (table 1). Among children aged <10 years who were born before 1 April 1993 and who were nevertheless vaccinated (according to their parents), the prevalences of HibPS antibodies (P < .002 for levels ≥1.0 μg/mL; P < .02 for levels <0.15 μg/mL) and the GMT (P < .05) were statistically significantly higher than those among children reported to be unvaccinated. A significantly higher prevalence of antibodies at levels ≥1.0 μg/mL was noted among those children aged <5 years who were born before 1 April 1993 and who attended a day-care center, in comparison with the prevalence among age-matched children who did not attend a day-care center (P < .01). For children born after 1 April 1993, no statistically significant difference was observed (data not shown).

Individuals with >1 child in their household who was attending a day-care center or elementary school had consistently higher antibody levels than did individuals without a child who was attending a day-care center or elementary school; the P value for the overall difference in prevalence at levels ≥1.0 μg/mL was...
mL was 0.06. The difference was more pronounced for women than for men (Table 1). The number of persons in the household did not have an effect on prevalence or GMT.

DISCUSSION

Persistence of antibodies after vaccination. We observed a sharp decline in the GMT within 6 months after infants had received the fourth vaccination as scheduled, but we noted 100% prevalence of HibPS antibodies at levels ≥1.0 μg/mL. After 2–2.5 years, the GMT seemed to have stabilized at 0.8 μg/mL; >70% of the children had HibPS antibody levels ≥1.0 μg/mL, and >80% of them had levels ≥0.15 μg/mL. We were unable to predict antibody levels for the long term after vaccination because of the relative newness of the conjugate Hib vaccine. Scheifele et al. observed better persistence after 4 PRP-T vaccinations of equal dosage: 72% of the vaccine recipients had antibody levels ≥1.0 μg/mL, and 99% had levels ≥0.15 μg/mL after 3 years [18]. However, a different vaccination schedule (vaccination at 2, 4, 6, and 18 months of age) was used, and their data were from a clinical trial, whereas our data concern vaccinations administered within a routine program. Furthermore, there are wide variations in antibody response that occur with the use of the same vaccine in different populations [19]. A key issue for the future is whether antibodies and memory immunity in vaccinated cohorts will persist for the long term.

The decline in HibPS antibody levels does not necessarily indicate a decline in immunity in vaccinated cohorts. HibPS antibody levels of ≥0.1–0.15 μg/mL are widely accepted as indicators of protection at the time of the assay. After vaccination with unconjugated PRP, subjects are considered to be protected for the long term when they have titers of ≥1.0 μg/mL [14, 15]. However, these cutoff values are not relevant to protection when we are evaluating the antibody response after vaccination with a Hib conjugate vaccine [15]. In contrast to nonconjugated vaccines, conjugated vaccines can elicit a T cell–dependent immune response; therefore, low or undetectable antibody levels do not necessarily indicate a lack of protection against invasive Hib infections.

This immunologic memory was shown by dramatic increases in antibody levels upon reexposure (colonization with Hib or...
cross-reacting organisms or vaccination with unconjugated PRP) in vaccinated children with previously undetectable antibody levels [20, 21]. Furthermore, in several European studies, the observed vaccine efficacy 1–3 years after conjugate Hib vaccination was much higher (87%–99%) than expected when 1.0 μg/mL (31%–40%) or even 0.15 μg/mL (62%–73%) was considered the protective level at 1 year after vaccination [15, 19, 22, 23].

Immunologic memory capacity is difficult to measure. Currently, this would entail obtaining 2 blood samples—taken before and after a booster dose of vaccine—from each participant, which would be a much greater burden on the participants. A laboratory test that could assess memory immunity in a single sample would be ideal.

**Unvaccinated cohorts.** The prevalence of HibPS antibodies and the GMT are high in the unvaccinated adult cohorts, but they did show a decrease with increasing age. When we interpret these cross-sectional data longitudinally, waning immunity seems to exist. It is unknown whether this is due to a decrease in functioning of the immune system with increasing age or to a decrease in contact with Hib and cross-reacting bacteria that boost HibPS antibody levels [24]. The latter explanation is supported by our finding that adults with children in their households had higher antibody levels than did adults without children in their households.

We observed a void in the immunity of children born just before Hib vaccination was introduced (children aged 3–7 years). This is due to the manner of introduction of vaccination; no catch-up vaccination campaign was established for older children. Meanwhile, circulation of the bacteria did probably decrease. Therefore, these children had a smaller chance of exposure to the bacteria and acquisition of natural immunity.

### Table 1. Prevalence of antibodies to the capsular polysaccharide of *Haemophilus influenzae* (at levels of <0.15 μg/mL, 0.15–0.99 μg/mL, and ≥1.0 μg/mL) and the geometric mean titer (with 95% CIs), in relation to possible associative factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percentage (95% CI) of patients with indicated antibody level</th>
<th>Geometric mean titer (μg/mL, 95% CI)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, 0–79 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>54.3 (50.7–57.9)</td>
<td>34.5 (31.7–37.4)</td>
<td>11.1 (9.5–12.7)</td>
</tr>
<tr>
<td>Women</td>
<td>56.9 (53.5–60.3)</td>
<td>33.5 (31.1–35.9)</td>
<td>9.6 (7.7–11.4)</td>
</tr>
<tr>
<td>All</td>
<td>56.2 (53.0–59.4)</td>
<td>33.6 (31.4–35.7)</td>
<td>10.2 (8.6–11.8)</td>
</tr>
<tr>
<td>Children born before 1 April 1993 (aged 2–9 y) whose parents reported their vaccination against Hib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>62.6 (51.1–74.1)</td>
<td>27.4 (16.6–38.2)</td>
<td>10.0 (2.3–17.7)</td>
</tr>
<tr>
<td>No</td>
<td>41.1 (33.9–48.3)</td>
<td>37.9 (31.8–44.0)</td>
<td>21.0 (16.1–25.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>36.1 (20.3–51.8)</td>
<td>40.4 (24.9–55.8)</td>
<td>23.6 (10.0–37.1)</td>
</tr>
<tr>
<td>Number of persons in households of children born before 1 April 1993 (aged 2–9 y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>50.4 (43.3–57.4)</td>
<td>32.5 (27.2–37.8)</td>
<td>17.1 (12.5–21.7)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>46.5 (39.9–53.1)</td>
<td>38.4 (31.7–45.0)</td>
<td>15.1 (10.5–19.7)</td>
</tr>
<tr>
<td>Children born before 1 April 1993 (aged 2–4 y) who attend a day-care center</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>48.8 (37.3–60.4)</td>
<td>33.3 (23.8–42.8)</td>
<td>17.9 (9.6–26.2)</td>
</tr>
<tr>
<td>No</td>
<td>22.8 (5.2–40.4)</td>
<td>62.3 (42.5–82.1)</td>
<td>14.9 (1.0–28.8)</td>
</tr>
<tr>
<td>Persons (aged 25–44 y) with children in the household who attend a day-care center or primary school</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>Yes</td>
<td>58.1 (51.2–65.1)</td>
<td>33.3 (26.6–40.0)</td>
</tr>
<tr>
<td>No</td>
<td>53.2 (47.8–58.6)</td>
<td>37.8 (32.6–42.9)</td>
<td>9.1 (5.5–12.6)</td>
</tr>
<tr>
<td>Women</td>
<td>Yes</td>
<td>62.7 (56.7–68.7)</td>
<td>31.0 (25.3–36.7)</td>
</tr>
<tr>
<td>No</td>
<td>54.0 (47.0–61.0)</td>
<td>37.1 (30.1–44.1)</td>
<td>8.9 (5.1–12.6)</td>
</tr>
</tbody>
</table>

**NOTE.** Hib, *Haemophilus influenzae* type b.
This is confirmed by the fact that those children who were born before 1 April 1993 but whose parents reported that they had been vaccinated had a higher antibody level than did their unvaccinated counterparts. A similar phenomenon was observed in other studies [25–27].

Another consequence of the lack of a catch-up vaccination campaign was the lack of herd immunity in The Netherlands during the first years after mass vaccination was started. In other words, in 1995, there was no visible, clear-cut decrease in the number of Hib isolates that were sent to the national reference laboratory and that were recovered from children born just before introduction of vaccination and from infants yet to be vaccinated [28]. However, the number of current Hib isolates from unvaccinated cohorts has declined by ~50%, in comparison with the numbers in the early 1990s [1], and it may decrease even further in the coming years. In other countries, herd immunity was observed immediately after a catch-up vaccination program [29]. In Finland, herd immunity was quickly observed, even though there was no catch-up campaign, probably because the force of infection was lower than that in our country [30].

Sociodemographic predictors of HibPS antibodies. The data from immunosurveillance studies on predictors of prevalence of HibPS antibodies are few, but risk factors for invasive Hib disease may also be correlated with higher antibody levels as a result of more frequent exposure to Hib. As in some other countries, the incidence of invasive Hib disease in The Netherlands has traditionally been slightly higher for men than for women, a fact that correlates well with the minimally lower antibody levels we observed in men [1, 6, 31].

In addition to vaccination, we found that attendance at a day-care center is a predictor of a higher level of HibPS antibodies in the prevaccination cohort. This finding is in accordance with an increased risk of invasive Hib disease found in other studies [6, 25, 26]. Istre et al. noted an increased risk of invasive H. influenzae disease among individuals with school-aged household members, which is in accordance with the higher antibody levels that we observed in individuals who had children in their households who attended a day-care center or primary school [7]. Extreme crowding (defined as ≥1.0 individuals per room) has been identified by Cochi et al. as an independent risk factor for invasive Hib disease [6]. Our variable number of individuals in the household was probably not a good proxy for crowding: household size was strongly correlated with crowding in the study mentioned, but it demonstrated no independent association with risk of Hib disease in a multivariate model. It is unfortunate that we did not have information on whether the participating infants were breast-feeding, since a number of studies [6, 32] have shown that breast-feeding protects against invasive Hib disease.

CONCLUSION

On the basis of this serosurveillance study and the sharp decline in invasive Hib disease after the introduction of vaccination, we conclude that the overall immunity in the Dutch population seems satisfactory. However, depending on the nature of the pathogen, a catch-up campaign may be necessary when a new vaccine is introduced, to prevent a void in immunity in children who have just missed the new vaccine and who subsequently have a smaller chance of acquiring natural immunity. Furthermore, the key questions for the future are whether Hib and cross-reacting organisms will circulate sufficiently enough to provide periodic natural reexposure and how long memory immunity will persist after vaccination without reexposure. Therefore, surveillance of invasive Hib disease in relation to vaccination status continues to be extremely important.

Acknowledgments

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References

11. van Gageldonk PGM, Mariani M, Berbers GAM. A comparison of ELISA methods for the determination of human serum antibodies to


