Decreased Point Prevalence of *Haemophilus influenzae* Type b (Hib) Oropharyngeal Colonization by Mass Immunization of Brazilian Children Less Than 5 Years Old with Hib Polyribosylribitol Phosphate Polysaccharide–Tetanus Toxoid Conjugate Vaccine in Combination with Diphtheria-Tetanus Toxoids–Pertussis Vaccine

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A protective herd effect has been described after susceptible populations of children are vaccinated with conjugate *Haemophilus influenzae* type b (Hib). Hib carriage was studied in children aged 6–24 months attending day care centers in two cities in southern Brazil (Curitiba and Porto Alegre). In Curitiba, routine immunization with Hib polyribosylribitol phosphate polysaccharide–tetanus toxoid conjugate vaccine (PRP-T) in combination with diphtheria-tetanus toxoids–pertussis vaccine (PRP-T/DTP) has been offered since September 1996; DTP vaccine alone is routinely given in Porto Alegre. Children in Porto Alegre ($n = 643$) were 8 times less likely to have received adequate Hib vaccination and 4 times more likely to be Hib carriers than children in Curitiba ($n = 647$; i.e., point prevalence of oropharyngeal colonization, 4.8% vs. 1.2%). Point prevalence of carriage with non–type b or other nontypeable Hi was similar in children of both cities. There was a vaccination effect on carriage rates in children who received a primary 3-dose series, independent of the booster dose, suggesting that a booster may be unnecessary to induce population protection.

Earlier studies showed that cases of *Haemophilus influenzae* type b (Hib) invasive disease decrease sharply after Hib vaccination has been introduced into a susceptible population [1–7]. Although some variation occurs between different vaccine formulations, conjugate vaccines against Hib protect against invasive disease with an efficacy of 94%–100% [2, 5–7]. As a consequence, Hib disease has been nearly eliminated in countries where widespread Hib conjugate vaccination has been adopted [8]. In the United States, the incidence of Hib disease among children began to drop after a polysaccharide–protein conjugate vaccine was introduced in 1988 for use among children aged 18 months. Over the following years, many polysaccharide–protein conjugate vaccines became widely used, and between 1987 and 1995 the incidence of invasive Hib disease among US children aged <5 years declined by 96% (from 41 to 1.6 cases per 100,000 children) [4, 9]. A decrease in the circulation of the pathogen appears to play an important role in disease control. Moreover, unvaccinated children up to age 5 years showed oropharyngeal Hib carriage rates of 3%–6% [1, 10–12], whereas carriage rates among Hib conjugate vaccinees were substantially lower (range, 0%–1.5%) [11–13].

In Brazil, Hib vaccination is not yet part of the national immunization program, although some cities widely use Hib vaccine. In Curitiba, the capital of the state of Parana, in mid-September 1996, routine immunization was introduced with lyophilized Hib vaccine consisting of purified polyribosylribitol phosphate polysaccharide conjugated to tetanus toxoid, reconstituted with diphtheria–tetanus toxoids–pertussis vaccine in a single syringe (PRP-T/DTP). Vaccine was given at ages 2, 4, and 6 months, with a booster dose administered at age 15 months. One year after introduction of routine immunization with PRP-T/DTP, the incidence of Hib meningitis among children aged <5 years in Curitiba decreased from 35.4 (1996) to 9.7 (1997) cases per 100,000 [14].

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Informed consent allowing each subject to participate in the study was signed by one of each child's parents. For children in foster care, informed consent was signed by the staff member who was their legal guardian within the institution. The study protocol was approved by the local ethics committee of the Federal University of São Paulo before initiation; procedures complied with the latest version of the Declaration of Helsinki.

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In the present study, we compared the point prevalence of oropharyngeal Hib colonization (i.e., carriage) among groups of vaccinated and unvaccinated children who attended public day care centers in two cities in southern Brazil, Curitiba and Porto Alegre. The city of Porto Alegre follows national immunization recommendations set by the Ministry of Health, based on the World Health Organization's Expanded Programme of Immunization for developing countries (i.e., immunization against bacille Calmette-Guérin at age 0–1 month; hepatitis B at ages 0, 1, and 6 months; diphtheria-tetanus toxoids–pertussis [DTP] and oral poliovirus at ages 2, 4, and 6 months; and measles at age 9 months), plus measles-mumps-rubella vaccine and booster doses of DTP and oral poliovirus during the second year of life. Curitiba differs in that DTP vaccine is replaced by a PRP-T/DTP combination. Curitiba and Porto Alegre were chosen as investigation sites because of their similar incidences of invasive Hib disease prior to Hib vaccine implementation and because of the striking difference in Hib vaccination coverage rates among children younger than 5 years of age (i.e., 99% in Curitiba and <10% in Porto Alegre). In addition, the two cities have similar population demographics and weather conditions.

Materials and Methods

Enrollment. From October to December 1997 (during the Brazil spring) and just 1 year after introduction of Hib vaccination in Curitiba, boys and girls ages 6–24 months who attended municipal day care centers in Curitiba and Porto Alegre were recruited (table 1), and specimens were collected. A typical day care center attended by children in this study divided children into separate rooms according to age, with ≤15 children in each room. Only children living in a household with other children ages 3–10 years (hypothetically being more prone to Hib exposure) were eligible for study participation. Children were excluded from the study if they had taken antibiotics up to 1 week before enrollment, were breast-feeding and their mother had taken antibiotics up to 1 week before the study, had a febrile illness, or did not fulfill any of the inclusion criteria described above. Children who were exposed to antibiotics were excluded because of contradictory data in the literature regarding the influence of antibiotic use on Hib oropharyngeal carriage. Children with febrile illnesses were excluded mainly to avoid the possible temporal association between events attributable to the ongoing disease and the specimen collection, which could cause the parents some degree of distress and suspicion regarding the safety of the procedure.

Children <1 year of age were considered adequately immunized against Hib if, prior to the study, they had received 3 doses of vaccine (when immunization began before age 6 months) or 2 doses (when immunization began between 7 and 12 months of age). For those vaccinated after age 12 months, a single injection was considered satisfactory. These definitions were used for data entry and statistical analysis.

Because routine Hib vaccination was introduced in Curitiba in mid-September 1996, 1 year before the study began (October 1997), these groups of children were likely to have received the following number of Hib vaccine doses: children aged 6 months (born in April 1997), 2 or 3 doses of the primary series; aged 7–14 months (born between August 1996 and March 1997), 3 doses; aged 15–18 months (born between April and July 1996), 1 or 2 doses of the primary series plus a booster dose when they reached the appropriate age; and aged 19–24 months (born between October 1995 and March 1996), a single booster dose at age 15 months (Hib vaccination was introduced after they were 6 months old). A small proportion of the children aged 15–24 months, who may have had access to immunization through private practitioners prior to public introduction of Hib vaccine, may have received up to 4 doses. Likewise, a small proportion of children enrolled in Porto Alegre received from 1 to 4 doses of vaccine, depending on their age, in private immunization clinics (the only access to Hib vaccination in that city).

Table 1. Demographic characteristics of the cities studied for *Haemophilus influenzae* type b (Hib) oropharyngeal colonization in Brazilian children <5 years of age.

Demographic characteristic	Curitiba	Porto Alegre
Total population	1,400,000	1,300,000
Live births (1996)	25,000	24,000
Incidence of Hib meningitis (cases/100,000) in children <5 years old (1995)	27	21
Hib vaccine coverage rate in children <5 years old (%)		
1996	<10	<10
1997	99	<10

Specimen collection. Prior to study initiation, 2 public health nurses from each city were trained to take oropharyngeal swabs. During the 8-week enrollment/collection phase, the local pair of nurses responsible for specimen collection was always assisted by 1 nurse from the other city, to avoid sampling bias. After swab sampling, the material was immediately placed in Stuart transport medium and given a coded identification number. On the same day, samples were sent by air to a reference microbiology laboratory at the Santa Casa de Misericórdia de São Paulo Hospital. The maximum time that elapsed between collection and inoculation on Hib-specific culture medium was 24 h.

Microbiology. At the microbiology laboratory, the swabs were inoculated onto selective media: 10% horse blood–chocolate brain-heart infusion agar plate (Difco Laboratories, Detroit) supplemented with 300 mg/L bacitracin [15]. Up to 5 suspected colonies per plate were studied. The isolates were identified through Gram's staining, recognition of typical morphology on chocolate agar plates, a requirement of NAD for growth by satellite phenomenon on ordinary blood agar, and by inability to convert γ-aminolevulinic acid (Sigma, St. Louis) to porphyrins. Fermentation of glucose, sucrose, lactose, xylose, and mannose in phenol red broth base (Difco) were performed for species identification [15, 16]. Capsular serotyping was done by slide agglutination with antisera to *H. influenzae* types a–f (Difco).

Statistics. A standard form containing data regarding birth date, sex, ethnicity, number of siblings at home, number of classmates, number of rooms in the household, Hib vaccination status (based on immunization records), and other relevant information was completed by a study nurse for each study participant. An expected sample size of 643 subjects per study group was calculated

Table 2. Characteristics of the study population of children from two cities in southern Brazil according to age, sex, ethnicity, number of other children per class at the day care, and number of rooms at home.

Characteristic	Curitiba (n = 647)	Porto Alegre (n = 643)
Age (days), median (range)	498 (180–745)	515 (182–749
Sex		
Male	314 (48.5)	356 (55.4)
Female	333 (51.5)	284 (44.6)
Ethnicity[a]		
White	522 (80.7)	422 (65.6)
Other	125 (19.3)	221 (34.4)
Previous Hib vaccination[a]		
Adequate or partially adequate[b]	606 (93.7)	71 (11.0)
No. of children/class at day care center, median (range)	15 (10–26)	17 (3–50)
No. of rooms at home[c]		
<5	262 (40.5)	226 (37.0)
⩾5	385 (59.5)	385 (63.0)

NOTE. Data are no. (%) unless otherwise indicated.

[a] $P < .01$.

[b] Adequate vaccination: children aged <1 year, 3 doses when immunization began before age 6 months or 2 doses when immunization began between age 7 and 12 months; children aged >1 year, single dose. Partially adequate vaccination: children aged <1 year, no more than 2 doses when immunization began before age 6 months or no more than 1 dose when immunization began between age 7 and 12 months.

[c] Thirty-two children from Porto Alegre who lived in a foster care facility are not included in this item analysis.

assuming $P = .8$, $\alpha = .05$, and a 4% difference in Hib carriage rates between vaccinated and unvaccinated subjects. Oropharyngeal Hib carriage rates were compared between study groups by use of odds ratios (ORs) and χ^2 or Fisher's exact test. A subgroup of 32 children from a Porto Alegre foster care facility was excluded from the analysis of the number of siblings aged <8 years, number of rooms at home, and number of classmates.

Results

About 94% of the children enrolled in Curitiba had been previously vaccinated against Hib (i.e., adequately or partially immunized for their age), an 8-fold greater vaccination rate than in Porto Alegre (11%; $P < .01$). Oropharyngeal swabs were obtained from 1290 children (647 in Curitiba, 643 in Porto Alegre). Characteristics of the study population are described in table 2. In brief, there was no statistically significant difference between the children in the two cities in terms of median age, sex, number of classmates, or number of rooms at home. There was a significant difference in ethnicity distribution ($P < .01$), as there were 23% more white children among the subjects in Curitiba. According to data available from both municipalities, 10.1% and 7.5% of children attended public day care centers in Curitiba and Porto Alegre, respectively (E. M. C. P. Maluf and A. K. Pustai, personal communication).

Overall, the prevalence of Hib carriage was 3.0%. It was significantly greater among children in the low coverage city (Porto Alegre, 4.8%) than in the high coverage city (Curitiba, 1.2%; $P < .01$). The carriage prevalence of other capsulated (e.g., types a, c, and f) and nontypeable *H. influenzae* was similar among children in both cities (table 3). Relationships between the study variables and Hib carriage rates are shown in table 4. When children in both cities were considered together, adequately immunized children were 4 times less likely to be Hib carriers than partially vaccinated or unvaccinated children (1.2% [7/607] vs. 4.7% [32/683], respectively; $P < .001$). Among subjects in the low coverage city, carriage prevalence among partially vaccinated or unvaccinated children was 5.3% (31/583); in contrast, there were no carriers among adequately immunized subjects (0/60). A similar phenomenon was not observed with children from the high coverage city: prevalence of 1.0% (1/100) among partially vaccinated or unvaccinated children versus 1.3% (7/547) among adequately immunized subjects. Point prevalence Hib carriage rates among adequately immunized children in the two cities were not significantly different (Fisher's exact test, $P > .999$).

When subgroups in the low coverage city were compared, there was an increased risk for Hib carriage (OR, 2.25; $P < .05$) in children with ⩾1 sibling aged <8 years: 3.2% (11/348) for no siblings versus 6.8% (18/263) for ⩾1 sibling. However, among children in the high vaccine coverage city, the number of siblings had no influence on the carriage rates: 1.4% (4/295) for no siblings versus 1.1% (4/352) for ⩾1 sibling.

Discussion

A recent review of the epidemiology of Hib disease in Latin America and the Caribbean estimated that the overall incidence of Hib meningitis among children aged 0–4 years is 35 cases per 100,000 children [17]. Large-scale Hib vaccination programs are rare in this region, and their effect has not yet been evaluated sufficiently. Nevertheless, data from Chile [6] and Uruguay [18] show that the inclusion of conjugate Hib vaccine in immunization programs in South American countries can be as effective as in the programs implemented in North America and Europe.

Reducing transmission may play an important role in Hib disease control. Unlike immunization with plain polysaccharide Hib vaccine (PRP), which does not affect carriage rates [1], the

Table 3. Frequency distribution of capsulated *Haemophilus influenzae* isolated from oropharyngeal swabs in children from day care centers in two cities, Curitiba and Porto Alegra, in southern Brazil, with high and low vaccination coverage, respectively.

Haemophilus serotype	Curitiba (n = 647; high coverage)	Porto Alegre (n = 643; low coverage)
a	1 (0.15)	2 (0.31)
b[a]	8 (1.2)	31 (4.8)
c	2 (0.31)	1 (0.16)
f	4 (0.62)	4 (0.62)
Nontypeable	91 (14.1)	76 (11.8)

NOTE. Data are no. (%).

[a] $P < .01$.

Table 4. *Haemophilus influenzae* type b (Hib) carriage distribution according to previous Hib vaccination, number of siblings <8 years old, number of rooms in the household, and number of classmates at the day care center in two southern Brazil cities, Curitiba and Porto Alegre, with high and low Hib vaccination coverage.

	Curitiba (high coverage)		Porto Alegre (low coverage)		Both cities	
Characteristic	Hib positive (%)	Total	Hib positive (%)	Total	Hib positive (%)	Total
Vaccination status						
Adequately immunized for age	7 (1.3)	547	0	60	7 (1.2)	607
Partially immunized for age	1 (1.7)	59	1 (9.1)	11	2 (2.9)	70
Unvaccinated	0	41	30 (5.2)	572	30 (4.9)	613
Siblings <8 years old[a]						
None	4 (1.4)	295	11 (3.2)	348	15 (2.3)	643
>1	4 (1.1)	352	18 (6.8)	263	22 (3.6)	615
Rooms at home[a]						
>5	4 (1.5)	262	11 (4.9)	226	15 (3.1)	488
<5	4 (1.0)	385	18 (4.7)	385	22 (2.8)	774
Classmates in day care[a]						
<15	3 (1.5)	194	19 (4.9)	391	22 (3.8)	585
>15	5 (1.1)	453	10 (4.5)	220	15 (2.2)	673

[a] Not included in analysis, 32 children from Porto Alegre who lived in a foster care facility.

use of conjugate vaccines is associated with a decrease in prevalence of Hib carriage among vaccinees [11–13, 19, 20], which will reduce transmission to and exposure of nonimmune persons [21].

In this point prevalence study in two cities in southern Brazil, rates of oropharyngeal Hib carriage correlated inversely with Hib vaccine coverage levels. Children from Porto Alegre, who were 8 times less likely to have received adequate Hib vaccination, were 4 times more likely to be Hib carriers (4.8%) than children from Curitiba (1.2%). Although the children in both cities lived under similar geographic, climatic, and socioeconomic conditions and shared most demographic characteristics, there was a significant difference in ethnicity. Although some reports suggest that ethnic differences reflect socioeconomic risk factors [22], the subjects in our study all attended public day care centers, suggesting that their families were nearly exclusively low income.

It is possible that the methods used for Hib isolation in our study slightly underestimated Hib oropharyngeal carriage rates, either by decreasing the yield by not using antiserum agar or by overgrowth of gram-negative organisms consequent to the delayed inoculation. Nevertheless, there is no reason to believe that this limitation would invalidate the comparison of Hib carriage rates between the two cities in the study.

Although the point prevalence in adequately vaccinated children from Curitiba and Porto Alegre did not differ significantly, evidence for herd protection was found only among the subjects in Curitiba, where high Hib vaccination coverage was achieved. When only children from Porto Alegre were considered (where just 9.3% [60/643] were adequately immunized against Hib), there was a clear-cut difference in carriage between adequately immunized (0% carriage rate [0/60]) and partially vaccinated/unvaccinated subjects (5.6% carriage rate [31/583]). Lower carriage rates, when compared with those of children in Porto Alegre, were observed among all Curitiba subgroups (see table 4), suggesting that the protective effect extended to all subjects, regardless of vaccination status. Published studies suggest that unvaccinated children who have little exposure to Hib are unlikely to be Hib carriers. For example, the Hib carriage rate tends to be lower among unvaccinated siblings of vaccinees than in other unvaccinated persons [11]. The data from Porto Alegre coincide with the results of *H. influenzae* carriage studies in both the Dominican Republic and Costa Rica, which evaluated children belonging to roughly the same age group, demonstrating an association between increased carriage and the presence of another child in the household [23, 24].

While our results seem to indicate a clear effect of Hib vaccination on carriage rates in nonvaccinated children in a high coverage situation, the actual effect on infection rates is not necessarily so clear-cut. Two good examples come from data collected in The Netherlands [25] and Alaska [26]. In The Netherlands, children born before the start of nationwide vaccination (April 1993) did not receive Hib vaccination. Although the incidence of Hib cases decreased sharply among vaccinated children during the following 3 years, the number of expected cases in unvaccinated children did not decrease. In Alaska, the incidence of invasive Hib disease decreased dramatically after the introduction of vaccination with Hib conjugate vaccine polyribosylribitol phosphate *Neisseria meningitidis* outer membrane protein vaccine. However, when the vaccination regimen was changed to diphtheria-tetanus toxoids–whole cell pertussis vaccine conjugated with Hib oligosaccharide CRM197 (DPT-HbOC), which is apparently less protective after the first dose to the youngest children, the number of Hib invasive cases increased, suggesting ongoing carriage and transmission in the population. Carriage rates among this population were 2.2%–13.2% (mean, 9.3%), and higher rates were associated with overcrowding and increasing age. Both studies suggest that

elimination of Hib circulation might not be achieved if the vaccination schemes adopted do not have a direct and age-extended effect on carriage rates.

Although the subgroup of 32 foster care residents from Porto Alegre had a particularly high Hib carriage point prevalence (6.3%), they were not excluded from the comparisons of Hib carriage point prevalence between children from Porto Alegre and Curitiba or between adequately immunized and partially vaccinated or unvaccinated children. They were excluded from calculations related to living conditions, where they did not fit in a specific category (e.g., siblings <8 years old, number of rooms in the household). Even if these subjects had been excluded from the other calculations, the 2 additional Hib carriers in this subset would not have changed the significant differences seen between the Hib carriage point prevalence in Curitiba and Porto Alegre and between adequately immunized and partially vaccinated or unvaccinated children (data not shown).

Carriage rates for other typeable *H. influenzae* did not differ between children from Curitiba and from Porto Alegre, showing the specificity of Hib vaccination. This observation is in accordance with other reports that demonstrated that introduction of large-scale Hib vaccination does not result in the increase of non–type b or other nontypeable *H. influenzae* diseases, as was feared would happen if the niche occupied by Hib colonization in the oropharynx was replaced by similar pathogens [9, 27]. Another important consideration is that the effect of vaccination on carriage rates observed in this study occurred in a population that had received either a primary 3-dose series without a booster (children aged <1 year) or a single dose of Hib vaccine (children in the second year of life). This could encourage administrators of vaccination programs in developing countries, where financial resources are scarce, to apply schedules that do not depend on a booster dose.

In summary, this study shows that widespread immunization with a Hib conjugate vaccine is associated with significant reduction in the number of Hib oropharyngeal carriers among Hib-immunized children. In a population receiving wide coverage with a Hib conjugate vaccine, the decrease in Hib carriage also was extended to nonimmunized children. In addition, our data provide support for the concept that it may be possible to achieve a population-protective effect with only a primary immunization schedule with Hib conjugate vaccine, without a booster dose. The protection of individuals provided by satisfactory and persistent anti-Hib polysaccharide antibody concentrations leads to lower rates of carriage, which is a protective mechanism for populations because of diminished Hib circulation and lower rates of transmission.

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