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Impact of vaccination against *Haemophilus influenzae* type b with and without a booster dose on meningitis in four South American countries

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ABSTRACT

To inform World Health Organization recommendations regarding use of *Haemophilus influenzae* type b (Hib) vaccines in national immunization programs, a multi-country evaluation of trends in Hib meningitis incidence and prevalence of nasopharyngeal Hib carriage was conducted in four South American countries using either a primary, three-dose immunization schedule without a booster dose or with a booster dose in the second year of life. Surveillance data suggest that high coverage of Hib conjugate vaccine sustained low incidence of Hib meningitis and low prevalence of Hib carriage whether or not a booster dose was used.

1. Introduction

Haemophilus influenzae type b (Hib) remains an important cause of bacterial meningitis and pneumonia among children, especially in countries that have yet to introduce highly effective conjugate vaccines [1]. The World Health Organization (WHO) recommends that all countries introduce Hib conjugate vaccine into their routine infant immunization programs [2]. Hib vaccines are commonly given as a three-dose primary series at the same time as diphtheria-tetanus-pertussis (DTP) vaccination, and several combination vaccines are available [3]. Hib conjugate vaccine has been shown to be highly effective in preventing invasive Hib disease after a three-dose primary series [4–6]. However, resurgence of invasive Hib disease several years after introduction of a three-dose, accelerated (2, 3 and 4 months of age) Hib vaccination schedule in the United Kingdom raised concerns about the need for a booster dose for maintaining long-term immunity [7]. While national immunization programs in many industrialized countries administer a booster dose of Hib vaccine between 12 and 18 months of age, WHO's 2006 position paper on Hib vaccines noted that additional data were required to determine the need for booster doses in developing countries [2]. To provide additional data from developing countries, WHO requested an analysis of trends in Hib disease incidence in countries in the Americas Region that included Hib vaccination in their routine infant immunization schedule with and without a booster dose.

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In 2006, all countries and territories except Haiti in the Americas Region had included Hib vaccination in their national immunization programs [8]. Among 19 Latin American countries with routine Hib vaccination, most provided a three-dose series of Hib vaccine without a booster, while 5 countries (Argentina, Mexico, Panama, Uruguay and Venezuela) provided a booster dose at 15–18 months of age [9]. The impact of vaccination on cases of Hib meningitis has been shown using surveillance data from several Latin American countries [8,10–12]. To evaluate potential impact of use of a booster dose, we used surveillance data to compare trends in Hib meningitis incidence among children <5 years in four countries, two of which had a three dose immunization schedule with no booster (Chile and Colombia) and two of which had a three dose primary immunization schedule with a booster dose in the second year of life (Argentina and Uruguay). Surveys of nasopharyngeal carriage were conducted among children in Argentina and Colombia to compare prevalence of Hib colonization several years after introduction of Hib conjugate vaccines.

2. Materials and methods

2.1. Study design

In 2002, the World Health Organization commissioned a multi-country evaluation to compare trends in Hib meningitis in Latin American countries using a three-dose primary immunization schedule for Hib vaccination with and without a booster dose in the second year of life. A multi-country protocol ("Propuesta para una evaluación sistemática del impacto de los programas de vacunación contra *H. influenzae* tipo b (Hib) en países Latinoamericanos" [in Spanish, available from the authors]) was developed by one of the study authors (R.L.). The evaluation was coordinated by the Pan American Health Organization (PAHO).

Sites for conducting the multi-country evaluation were selected in two countries that used a three-dose schedule without a booster (Chile and Colombia) and two countries that used a three-dose primary schedule with a booster dose in the second year of life (Argentina and Uruguay, Table 1). Sites were selected based on the following criteria: a minimum population of 250,000 children <5 years of age, well-defined geographic area, reliable population estimates for specific age groups, good access to and utilization of health services, routine bacteriological investigation of suspected cases of meningitis, mandatory notification of Hib and other bacterial meningitis established at least two years prior to Hib vaccine introduction, bacteriological confirmation of at least 60% of reported cases of bacterial meningitis, a minimum of 5 years of Hib vaccination and coverage with three doses of Hib vaccine in the first year of life of at least 85%. Vaccination histories were requested for cases of Hib meningitis in vaccine-eligible children. Cases of Hib meningitis in children who completed the primary, three-dose immunization series were considered vaccine failures. Although only one year of surveillance data was available prior to vaccine introduction in Argentina, data from Argentina were included in this analysis due to the availability of several years of data from continuous surveillance following vaccine introduction.

2.2. Hib vaccination in study sites

All four countries have publicly financed national immunization programs that provide recommended vaccines at no charge to the population. Hib conjugate vaccines were introduced in Uruguay in 1994, Chile in 1996, Argentina in 1997 and Colombia in 1998. Introduction of Hib conjugate vaccines in Chile and Uruguay has previously been described [11,12]. At the time of introduction, Uruguay conducted mass vaccination of children 1–4 years of age with a single dose of Hib vaccine; the other countries introduced Hib vaccination without catch-up campaigns. Chile, Colombia and Uruguay began with monovalent Hib vaccines and then switched to combined diphtheria, tetanus, whole cell pertussis, Hib and hepatitis B (pentavalent) vaccine (Colombia in 2001, Chile in 2006 and Uruguay in 1999) [8]. Argentina used a combination diphtheria-tetanus-whole cell pertussis-Hib vaccine since introduction [8]. The childhood immunization schedules of all four countries recommended a three-dose, primary immunization schedule of Hib-containing vaccines at 2, 4 and 6 months of age. Schedules in Uruguay recommended a booster at 12 months and in Argentina at 18 months; Chile and Colombia did not recommend a booster [8]. Hib conjugate vaccines were available in the private sector in all four countries prior to their introduction in the national immunization program, although private purchase accounted for a small number of doses [8,11,12].

2.3. Surveillance for Hib meningitis

All four countries have national, laboratory-based surveillance for invasive bacterial diseases. Sterile-site *H. influenzae* isolates from patients with meningitis and other invasive syndromes are sent to national reference laboratories for identification and serotyping using standard methods. National reference laboratories in all four countries participated in a regional surveillance system for characterization of *Streptococcus pneumoniae*, *H. influenzae* and *Neisseira meningitidis* (*Sistema Regional de Vacunas*, SIREVA) [13], including an international quality assurance program [14]. For each country, surveillance areas were chosen for calculation of population-based incidence of Hib meningitis based on isolates received at national reference laboratories from surveillance area residents (Table 1). National census data were used to estimate population denominators for pre-vaccine base-line and post-vaccine study periods.

2.3.1. Uruguay

Uruguay maintains national, laboratory-based surveillance for Hib meningitis, which has been a notifiable disease since 1990. All *H. influenzae* isolates are sent to the national reference laboratory for serotyping.

2.3.2. Chile

In Chile, we used data from a previously published, retrospective assessment of pre-vaccine Hib incidence rates among children <5 years of age in the Metropolitan Region (Santiago), over a three-year period from 1985 to 1987 [15]. Between November 1992 and November 1993 (four years prior to introduction of Hib conjugate vaccine into the routine childhood immunization schedule), approximately 30,000 children (half of the birth cohort) in the surveillance area received 3 doses of Hib conjugate vaccine as part of a postlicensure vaccine trial [16]. Data for the post-vaccine study period (2003 through 2005) were provided by Centro para Vacunas en Desarrollo (CVD-Chile) based on laboratory-based surveillance for invasive Hib disease in the metropolitan region [17]. *H. influenzae* isolates were serotyped at the national reference laboratory.

2.3.3. Argentina

In Argentina, national surveillance for invasive Hib disease was implemented in 1996. In this analysis, we included Hib meningitis cases identified at hospitals in Buenos Aires metropolitan area, including the city of Buenos Aires and 10 surrounding cities, for which isolates were received by the national reference laboratory at the Instituto Nacional de Enfermedades Infecciosas.

Table 1
Characteristics of surveillance areas in four South American countries that contributed data on Hib meningitis incidence before and after Hib vaccine introduction for this study.

Surveillance area	Pop. <5 years	Type of surveillance	Year Hib conjugate vaccine introduced	Recommended number of doses (age in months)[a]	Hib coverage[b] <1 year (study period)
Countries with three-dose primary immunization schedule without booster dose					
Metropolitan Region (Santiago), Chile	507,625	Active, laboratory based	1996	3 (2, 4, 6)	92% (2003–2005)
Bogotá, Colombia	626,631	Retrospective review of laboratory records	1998	3 (2, 4, 6)	80% (2002–2004)
Countries with three-dose primary immunization schedule with booster dose					
Uruguay (national)	265,000	Passive, laboratory based	1994	3 + 1 (2, 4, 6, 12)	95% (2002–2004)
Buenos Aires metropolitan region, Argentina	3,344,817	Retrospective review of laboratory records	1997	3 + 1 (2, 4, 6, 18)	94% (2003–2005)

[a] Four-dose schedules (three-dose primary [infant] immunization with a booster dose in the second year of life) are shown as 3 + 1.
[b] Estimated coverage with 3 doses of Hib-containing vaccine by 12 months of age. *Source*: Pan American Health Organization.

2.3.4. Colombia

In Colombia, laboratory records were reviewed to identify Hib meningitis cases in persons <5 years at all hospitals in the city of Bogotá from 1995 through 2004. Data included in this study included Hib meningitis cases for which isolates were received by Instituto Nacional de Salud, the national reference laboratory.

2.4. Cross-sectional studies of Hib colonization

As part of the multi-country protocol, cross-sectional surveys of nasopharyngeal Hib colonization were conducted among children one to six years of age in Argentina, Chile and Colombia. The objective of the carriage surveys was to determine whether preschool-aged children (five years old) residing in countries using a booster dose had lower prevalence of Hib colonization than preschool-aged children in countries without a booster dose. Children 12 months of age were included to compare colonization rates following the primary, three-dose Hib vaccination series. Target sample size included 900 one-year old and 700 five-year-old children, based on estimated prevalence of Hib colonization of 1% (±0.6%) and 3% (±1.5%) among one- and five-year-old children, respectively. If the booster dose had little impact on Hib carriage among preschool-aged children, the prevalence of carriage was expected to be the same among younger and older children in the same population. Hib conjugate vaccines had been used for more than five years in all four countries at the time of the cross-sectional study of nasopharyngeal colonization.

In Argentina, children were enrolled between June 2005 and April 2006 at two pediatric hospitals in the city of Buenos Aires. In Colombia, children were enrolled between October 2005 and August 2006 at vaccination clinics in 7 public health facilities and one clinic serving the armed forces. Due to differences in the protocol used in Chile for the nasopharyngeal colonization survey, results from Chile were excluded.

Nasopharyngeal specimens were obtained by introducing a flexible calcium alginate swab through the nares to the back of the nasal cavity following standard procedures [18]. Swabs were rotated prior to removal, and placed immediately in skim milk–tryptone–glucose–glycine (STGG) transport media [19]. Inoculated transport medium was kept cool during transport to microbiology laboratories and processed within 6 h. Samples were vortexed for 10–15 s, 50 μl were plated on chocolate agar with 200 μg/ml bacitracin and plates were incubated at 37 °C with 5% CO_2 for 18–48 h. *H. influenzae* were identified by biochemical tests, characteristic colony morphology and requirements for X and Y factors. Isolates were serotyped using type-specific antisera (Difco; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and polymerase chain reaction [20].

2.5. Data analysis

Age-specific incidence rates were calculated by dividing the number of Hib meningitis cases by the population of each age group in the surveillance area. To account for substantial variability in pre-vaccine incidence rates, we compared age-specific incidence of Hib meningitis during the post-vaccine study period to incidence during the pre-vaccine base-line period in each country. Pre-vaccine base-line periods included three years of surveillance for Hib meningitis in Chile, Uruguay and Colombia, versus only one year in Argentina. We calculated relative incidence rates and 95% confidence intervals using EpiInfo (version 6.04d, Centers for Disease Control and Prevention, Atlanta, USA). For comparison with published data on the prevalence of nasopharyngeal colonization, we estimated the prevalence of Hib or any *H. influenzae* carriage by age group (one-year old or five-year-old children) among children enrolled in the carriage surveys in each country.

3. Results

3.1. Surveillance for Hib meningitis

Data provided by the four countries showed wide variation in Hib meningitis incidence among children <5 years during the pre-vaccine period, from 47.5 to 4.9 cases per 100,000 among children <1 year and 7.0 to 0.5 cases per 100,000 among 1–4 years (Table 2). Hib meningitis rates prior to vaccine introduction were highest in surveillance areas in Chile and Uruguay and lowest in Colombia. In Chile, Hib meningitis incidence among children <1 and 1–4 years in 1993 through 1995 was lower than during the pre-vaccine base-line period (Fig. 1).

Following Hib vaccine introduction, rates of Hib meningitis declined and were sustained at low levels through the study period in all four countries (Fig. 1). Incidence of Hib meningitis during the post-vaccine study period varied from 2.3 to 1.2 cases per 100,000 among children <1 year and 0.5 to 0 cases per 100,000 among 1–4 year olds (Table 2). Surveillance data from all four countries demonstrated that Hib meningitis cases continued to occur, albeit at low levels, 6–10 years following vaccine introduction. Contrasting Hib meningitis incidence during the post-vaccine period with the pre-vaccine base-line period, relative rates were similar in countries with and without booster doses (Table 2). Use of different post-vaccine periods did not change the results, as rates have remained

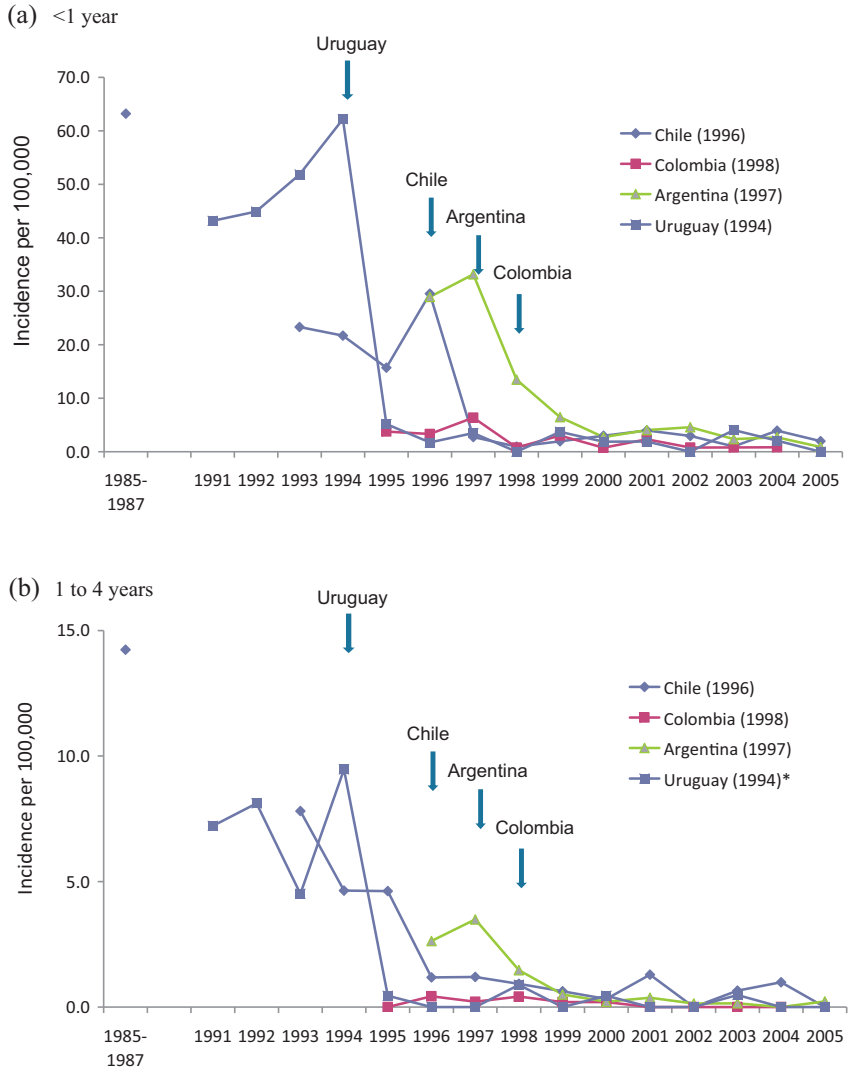

Fig. 1. Trends in Hib meningitis incidence in 4 South American countries before and after introduction of Hib vaccines in national immunization programs: (a) <1 year; (b) 1–4 years of age. *In 2005 the age groups used for reporting changed; from 2005 to 2009 the cases among children aged 48–59 are included in the 1–4-year-old group.

low following Hib vaccine introduction regardless of whether a booster dose was recommended (Fig. 1).

Data on vaccination status of Hib meningitis cases identified during the study period were provided by Chile and Colombia. In Chile, 9 of 10 children at least 6 months of age with Hib meningitis during 2003–2005 had received 3 doses of Hib vaccine (i.e., vaccine failures). In Colombia, two Hib meningitis cases occurred in children at least six months of age; neither had been vaccinated. No information was provided on underlying illnesses or immunodeficiencies among cases of vaccine failure.

3.2. Cross-sectional surveys of Hib colonization

In cross-sectional surveys of nasopharyngeal carriage conducted in two countries during 2005–2006, prevalence of Hib carriage was too low (<0.1%) to make comparisons between age groups or schedules. In Argentina, one (0.1%) of 900 one-year olds and none of 700 five-year-old children were colonized with Hib at the time of the survey, versus one (0.1%) of 916 one-year olds and none of 431 older children in Colombia. Prevalence of carriage of any *H. influenzae* was 44% among one-year olds and 36% among older children in Argentina, versus 33% among one-year olds and 28% among older children in Colombia. In both countries, more than 90% of *H. influenzae* identified were nontypeable (unencapsulated) organisms.

4. Discussion

Analysis of meningitis surveillance data from four Latin American countries showed significant reductions in Hib meningitis incidence through 6–10 years following vaccine introduction, whether or not a booster dose was given in the second year of life. In two countries without a booster dose, Colombia and Chile, high coverage with three vaccine doses sustained low incidence rates among children <5 years at least 6–9 years after vaccination. There have been no reports of increasing incidence of Hib disease since the study period in these countries or from other Latin American countries. While booster doses appear unnecessary for maintaining effective control of Hib meningitis for several years in these two countries, results should be interpreted cautiously. Although incidence declined significantly in all countries following introduction of Hib conjugate vaccine, low pre-vaccine incidence in Colombia compared to rates in other countries likely reflects difference in sensitivity of surveillance for Hib disease rather than geographic differences in incidence rates. Additional study of the need for booster doses in developing countries is warranted, especially in

Table 2
Comparison between age-specific rates of Hib meningitis in surveillance areas during the study period with rates during a base-line period prior to Hib vaccine introduction in four South American countries.

Surveillance area	Base-line period	Age group	Cases	Population	Rate per 100,000[a]	Study period	Total cases (no. of vaccinated[b])	Population	Rate per 100,000[a]	Relative rate (95% CI)
Countries with three-dose primary immunization schedule without booster dose										
Metropolitan Region (Santiago)[c], Chile	1985 –1987	<1 year	154	108,127	47.5	2003 –2005	7 (3)	101,351	2.3	0.05 (0.02, 0.10)
		1–4 years	88	420,815	7.0		6 (6)	406,273	0.5	0.07 (0.03, 0.16)
Bogota, Colombia	1995 –1997	<1 year	17	126,236	4.5	2002 –2004	3 (0)	123,060	1.2	0.18 (0.04, 0.65)
		1–4 years	3	477,325	0.2		0	508,412	0.0	ND
Countries with three-dose primary immunization schedule with booster dose										
Uruguay	1991 –1993	<1 year	81	57,887	46.6	2002 –2004	3	49,597	2.0	0.04 (0.01, 0.14)
		1–4 years	44	221,721	6.6		1	208,910	0.2	0.02 (0.00, 0.18)
Buenos Aires[c], Argentina	1996	<1 year	201	694,128	29.0	2003 –2005	40	668,963	2.0	0.21 (0.15, 0.29)
		1–4 years	73	2,776,510	2.6		10	2,675,854	0.1	0.14 (0.07, 0.28)

[a] Total number of cases per 100,000 population per year in specified age group.
[b] Cases of Hib meningitis with 3 documented doses of Hib vaccine.
[c] Includes cities in the metropolitan region.

populations with high HIV prevalence and in geographic regions with different patterns of Hib disease.

Concerns about use of a three-dose immunization series without a booster for long-term control of Hib disease arose following resurgence of invasive Hib disease several years after introduction of Hib vaccination schedule in the United Kingdom. A unique set of circumstances may have contributed to this resurgence of Hib disease [21,22]. The United Kingdom introduced Hib vaccination in 1992 with a three-dose, accelerated primary infant series, with doses at 2, 3, and 4 months of age. A massive catch-up vaccination campaign was conducted among children 12–48 months of age, leading to sharp declines in cases of invasive Hib disease [23]. In the four South American countries in this analysis, only Uruguay vaccinated older children with catch-up vaccination. In the United Kingdom, a temporary shortage of diphtheria-tetanus-whole cell pertussis-Hib combination vaccines resulted in children receiving a less immunogenic Hib conjugate vaccine formulation in combination with diphtheria-tetanus-acellular pertussis vaccines [24]. Lack of a booster dose of the combination vaccine containing acellular pertussis may have led to increased Hib colonization. Notably, the four Latin American countries included in this analysis used combination vaccines containing whole-cell pertussis antigens throughout the study period. In the UK, waning immunity following low levels of transmission led to increased susceptibility in all age groups [7]. Hib disease affected children who had received a complete three-dose primary Hib series as well as adolescents and adults who had never been vaccinated. Following introduction of a booster dose and catch-up vaccination for children >1 year of age, Hib disease declined again to low levels [25]. Increased rates of Hib disease have also been observed in countries that did not use acellular pertussis-containing vaccines [26,27], suggested that booster doses may be needed regardless of vaccine formulation to maintain long-term immunity and sustain low levels of Hib disease many years after vaccination.

In low income countries and specific populations with high rates of Hib disease, such as Native American, Alaskan Native and Aboriginal peoples, the epidemiology of Hib disease is marked by early nasopharyngeal colonization and a peak in the incidence of invasive disease between 6 and 18 months of life [28]. For this reason, a three-dose primary immunization series, begun as early as six weeks of age, is recommended to prevent severe Hib disease in infants and young children. A three-dose immunization schedule without a booster successfully maintained low incidence of invasive Hib disease among young children in The Gambia more than 10 years after vaccine introduction [4]. However, cases in older children and vaccine failures were identified in the same population, showing that children are still being exposed to Hib [27]. In settings of high vaccination coverage with adequate surveillance for invasive Hib disease, some cases in completely vaccinated children should be expected. However, if vaccination shifts susceptibility to older age groups, booster doses may be needed to prevent disease in older children.

Countries in Latin America were among the first low and middle income countries to introduce Hib conjugate vaccines into national immunization programs [29]. Hib vaccine use has increased dramatically worldwide in the past few years and is nearly universal among countries in the Americas [8,30]; most developing countries have adopted a three-dose immunization series without a booster dose [31]. Among four "early adopting" countries, Chile was the only country to introduce Hib vaccine without a booster [12], based on economic factors as well as results of a trial introduction [17]. Several countries in the Americas added booster doses to take advantage of combination vaccines including diphtheria, tetanus and pertussis. In Chile, due to a temporary shortage of diphtheria-tetanus-whole cell pertussis vaccine in 2009, the national immunization program switched to a pentavalent

combination vaccine with DTP, Hib and hepatitis B at 18 months of age. In many countries in the Americas and other regions that provide booster doses of DTP vaccine in the second year of life, price of vaccine has been the main obstacle to introduction of Hib booster doses, rather than concern about adding a vaccination visit in the second year of life [9].

The surveillance data included in this analysis were subject to many limitations. Ideally, evaluations of the need for booster doses should include data for all invasive syndromes, not just meningitis. Meningitis accounts for a small proportion of invasive Hib disease and is most common in the first year of life [1]. The multi-site protocol was limited to comparisons of incidence rates for Hib meningitis because surveillance was not conducted in all countries for other invasive *H. influenzae* syndromes, such as pneumonia and sepsis. Prevention of syndromes that occur after the first year of life may depend more on booster doses and therefore present better endpoints for evaluation. Secondly, laboratory-based surveillance for Hib disease should be well-established in the country several years prior to vaccine introduction. Serotyping is required to verify that isolates of *H. influenzae* are Hib. In Argentina, only one complete year of surveillance data was available prior to vaccine introduction. In Colombia, low rates of Hib meningitis prior to vaccine introduction suggested under-ascertainment of cases. Third, complete vaccination histories for cases of Hib disease are important to distinguish between vaccine failure and missed opportunities. In Colombia, none of the Hib meningitis cases were completely vaccinated, while in Chile, the majority of case patients had completed the primary immunization series. Analysis from one hospital in Chile showed that a substantial proportion of invasive Hib disease after vaccine introduction occurred in children old enough to have benefited from a booster dose [32]. Although booster doses of Hib conjugate vaccine may not have been required to maintain low incidence of Hib meningitis, use of a booster dose might have prevented Hib meningitis cases in fully vaccinated children older than one year of age.

Finally, we observed low prevalence of Hib colonization in two studies that conducted cross-sectional studies following the multi-country protocol. Identification of Hib colonies in cultures of nasopharyngeal flora is challenging and dependent upon laboratory technique [33], and prevalence of Hib colonization may have been underestimated in these studies. However, low prevalence of Hib colonization has been reported from several countries following widespread vaccination, including in cross-sectional colonization surveys conducted in the United Kingdom prior to and during the resurgence of Hib disease [34]. We were not able to evaluate the effect of booster doses of Hib conjugate vaccine on nasopharyngeal carriage, which might presage a growing reservoir of Hib before increased rates of disease are detected [35,36].

In conclusion, surveillance data from these four South American countries with high coverage of Hib conjugate vaccine show that declines in Hib meningitis incidence have been sustained whether or not a booster dose was used. This analysis illustrates the need to maintain surveillance for Hib infections to evaluate long-term impact on disease incidence and inform decisions regarding use of booster doses. Since humans are the only reservoir for Hib, elimination of Hib is theoretically possible. However, elimination has been elusive even in countries with high vaccination coverage, including booster vaccination. The lessons learned from use of different schedules of Hib conjugate vaccines may prove useful as countries start using pneumococcal conjugate vaccine [37].

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