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Dynamics of natural immunity caused by subclinical infections, case study on *Haemophilus influenzae* type b (Hib)

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SUMMARY

Natural immunity to *Haemophilus influenzae* type b (Hib) is based primarily on antibodies that are thought to develop in response to subclinical infections. Wide use of conjugated Hib vaccines could lead to decreases in circulating Hib bacteria, thereby diminishing antibody levels in the unvaccinated. We applied a statistical model to estimate the duration of natural immunity to Hib under different forces of infection. Prior to the introduction of conjugated Hib vaccines, new Hib infections were estimated to occur once in 4 years and the antibody concentration to stabilize at a level around 1 μg/ml. In the absence of new stimuli, i.e. infection, 57% of the unvaccinated population would become susceptible to invasive disease (antibody levels $< 0{\cdot}15$ μg/ml) in 10 years. Due to an interaction between the force of infection and the duration of immunity, in some situations numbers of invasive infections could increase in unvaccinated cohorts. This theoretical scenario has yet to be observed in practice.

INTRODUCTION

Optimizing vaccination strategies is one of the major aims of infectious disease epidemiology. In order to assess the need, timing, and target groups for revaccination, estimation of the duration of vaccine-induced protection is of paramount importance. In addition, for some diseases, the existence and duration of natural immunity in unvaccinated cohorts is relevant especially when considering population-wide vaccination programmes. For *Haemophilus influenzae* type b (Hib), natural immunity acquired during childhood has been thought to explain the pattern of disease occurrence, with predominance in children less than 5 years of age [2]. Natural immunity, in turn, is believed to depend on repeated exposure to Hib bacteria resulting in production of functional antibody [2, 3]. As the wide use of Hib conjugate vaccines amongst children has been shown to reduce the carriage of Hib bacteria in vaccinated populations [4–8], concerns have been raised about the possibility of waning of natural immunity in unvaccinated cohorts including adults, adolescents and unvaccinated children [3, 9, 10].

Hib bacteria express a highly antigenic polysaccharide capsule. As a rule, polysaccharide antigens do not stimulate T lymphocytes required for the development of immunological memory. Therefore, secondary type responses and cell-mediated immunity are not seen and protection against invasive infection is mediated solely via antibodies. In addition to Hib, contacts with other bacteria with capsular polysaccharide antigenically similar to Hib have been suggested as a mechanism that may result in production of Hib specific antibodies [11–14]. Several studies have provided data suggesting that individuals are protected against invasive Hib disease if the concentration of serum antibodies is above $0{\cdot}15$ μg/ml [15–18]. A much higher level, 10 μg/ml, is assumed to be required for protection against acquisition of carriage, i.e. subclinical infection [4, 19, 20].

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Serum antibody concentrations have usually been measured soon after vaccination. However, duration of immunity also depends on the rate of decline of serum antibody concentration. Studies on passively administered IgG antibodies suggest that the half-life of Hib IgG antibodies is around 45 days [21]. For actively produced antibodies, the decline should ideally be studied by serological follow-up after disease or after vaccination. In practice, this may be complicated by repeated subclinical infections that may result in new antibody responses during the follow-up (cf. hepatitis B [22]). Studies on Hib antibody dynamics are particularly problematic since unobserved antigen stimuli due to subclinical re-infections are common. Only very high antibody concentrations (10 μg/ml) would prevent them [4, 19, 20].

In a previous paper we developed a model accommodating the possibility of unobserved antigen stimuli during the follow-up [1], enabling us to estimate simultaneously the rate of decline of antibody concentration and the incidence of subclinical infections. The results presented in this paper, obtained by using the same model, are intended to provide insight into the interplay between these two parameters, and to study how changes in the incidence of infection affect protective immunity in the unvaccinated population. The data used in the model were collected before Hib conjugate vaccines were available. The children studied were vaccinated at the age of 4–7 years, when their antibody responses to the Hib vaccine were already mature. We consider antibody responses to Hib capsular polysaccharide vaccine as a proxy to natural antibodies arising as response to subclinical Hib infection. Responses to polysaccharide vaccines have been extensively studied and shown to be similar to natural responses to Hib carriage or disease both in magnitude and immunoglobulin isotype distribution [23–26]. The relevance of the results, both the estimates for the duration of natural immunity and the role of subclinical infections in it, is discussed in relation to the conjugated Hib vaccines currently in use.

MATERIALS AND METHODS

Data

The data consist of 418 follow-up measurements of Hib antibody from sera of 110 children vaccinated with the Hib polysaccharide vaccine in an efficacy trial in Finland during 1974–7 [23, 27]. The children received the polysaccharide vaccine as a single injection at the age of 4–7 years. Following the injection, up to four serum samples were gathered at 1-year intervals. All samples were analysed simultaneously at the end of the study. There were 4 children with 2, 14 with 3 and 92 with 4 follow-up measurements.

Biological assumptions and definitions

Up to 5% of children in Finland harboured Hib in their nasopharynx at the time of the study [4]. However, Hib only rarely caused invasive disease; among children aged less than 5 years, the annual incidence was $52/10^5$ [28]. Earlier polysaccharide vaccine studies had suggested that individuals were protected against invasive disease if the concentration of serum antibodies was above 0·15 μg/ml [15–18]. In practice, only infections acquired while the antibody level is under this threshold may lead to invasive disease. As still only a fraction of these do progress to invasion we have designated such infections 'potentially serious'. A far higher antibody concentration, 10 μg/ml, is assumed to be required for protection against nasopharyngeal carriage, referred to from now on as 'subclinical infection' [4, 19, 20]. For the incidence rate of subclinical infections (infection pressure) we have used the term 'force of infection'. Invasive infections have been presumed to start as subclinical infections and have thus been included in this measure; however, their share of all infections is small.

We assumed that antibody responses to both Hib polysaccharide vaccine and subclinical Hib infection were similar. This assumption was based on the identical structure of the Hib polysaccharide vaccine and the polysaccharide capsule of Hib bacteria, and on data on antibody responses to Hib infection and to polysaccharide vaccine [10, 23–26]. The data we used did not contain measurements from children aged less than 4 years. Therefore our predictions, as well as the discussion points raised, apply only to cohorts above 2 years of age, those age groups generally accepted as being capable of responding well to polysaccharide antigens [10].

Model for the decline of antibody concentrations

In the dataset, there were a number of individuals whose Hib antibody titres did not decrease over the

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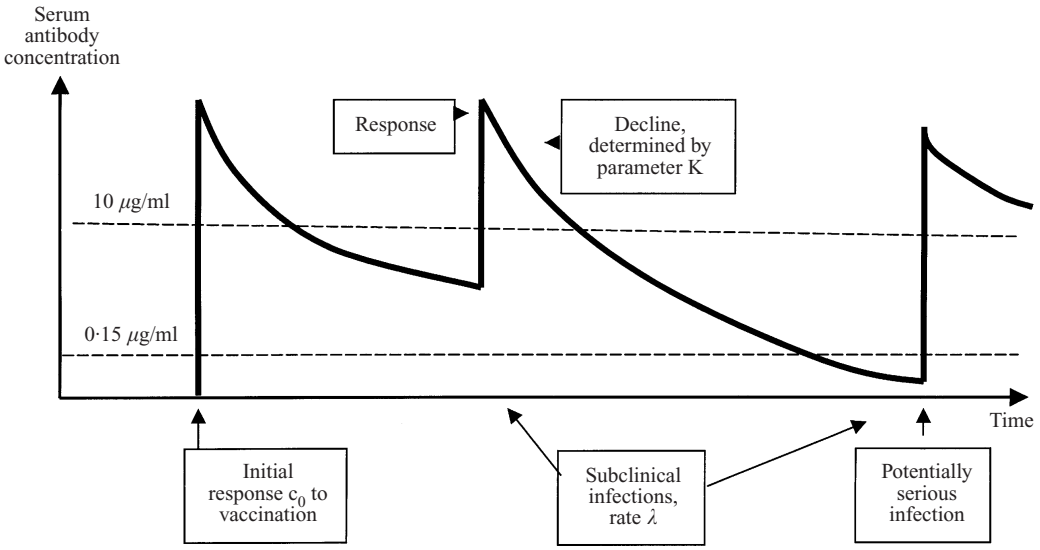

Fig. 1. Schematic illustration of Hib antibody dynamics, including an initial response (c_0), decline during the follow-up and occasional new responses via subclinical infections. The times of the responses are not directly observed (except the time t_0 of vaccination) but the sequence of responses and subsequent declines are 'fitted' to the data. Scales are logarithmic. Antibody levels 10 μg/ml (protection from subclinical infection) and 0·15 μg/ml (protection from invasive disease) are marked with dashed lines.

study period, following initial vaccination. It was assumed that the observed antibody concentration resulted not only from the magnitude of the response to initial stimulus (vaccination) and the subsequent rate of antibody decline, but was also affected by subclinical infections occurring between vaccination and antibody measurement. The statistical model used [1] included these three elements (Fig. 1).

The initial log-response ($\ln c_0$) to vaccination at time t_0 was assumed to be normally distributed. After the response, the antibody concentration $c(t)$ in the serum decreased according to:

$$\ln c(t) = \ln c_0 - K(t - t_0)^a.$$

Parameter K determines the rate of decline of the antibody concentrations. Heterogeneity in the decline was allowed by assuming that K is normally distributed across individuals. The dependence of antibody decline on time is regulated by parameter a. Value $a = 1$ would correspond to a model with exponential decay. The data did not support the exponential decay model, but was consistent with the chosen value of 0·5 for a (more discussion in [1]).

The model allowed subclinical Hib infections to occur at a rate of λ during the study period (Fig. 1). The magnitude of antibody responses to subclinical infection was assumed to have the same distribution as the response c_0 to vaccine (supported by data in references 23–26). Although such infections were not directly observed, their frequency could be inferred from the observed data via the statistical model.

The model involved a three-level hierarchy of: (*a*) model parameters (e.g. rate of subclinical infection), (*b*) unobserved quantities defining the antibody processes (curves) as depicted in Figure 1, and (*c*) the observed antibody measurements. A Bayesian hierarchical model was specified in terms of the sampling distributions of the antibody processes conditional on model parameters, and of the data conditional on the antibody processes [1]. We used novel numerical integration techniques (Markov chain Monte Carlo sampling) to estimate the model parameters [1].

Results from Bayesian analyses are expressed in terms of probability distributions for unknown model quantities (parameters and predicted observations). After having incorporated the evidence from the observed data into prior knowledge about the parameters, these so-called posterior distributions express the uncertainty that exists about the unknown quantities [29]. The prior knowledge is given in terms of a prior distribution; we used a prior that had minimal effect on the results. Inferences about the basic parameter values (e.g. the *population* mean and standard deviation of the initial response) were given in [1]. In this paper, we have summarized these results in terms of predictive distributions for *individual* observables (e.g. the mean and 90% interval for the initial response in an individual), and have produced

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new predictions regarding the interaction between immunity and force of infection. The results have been expressed as posterior predictive mean or median values of unknown quantities with 90% posterior predictive intervals (90% credible intervals, CI).

RESULTS

Force of infection and the speed of antibody decline

The estimated force of infection λ, i.e. the incidence of subclinical infection, implied an average of 0·25 infections per year per child (90% CI 0·12–0·39) and a mean time interval of 4·6 years between infections (90% CI 0·2–14). The risk of contracting a subclinical Hib infection during the 4 years of follow-up in the study material, using the mean value of 0·25 infections per year, was thus 0·63 ($= 1 - \exp[-4 \times 0{\cdot}25]$).

With zero force of infection ($\lambda = 0$), the relative decrease in antibody concentration is dependent only on the magnitude and variability of the decline rate (K) in the study population (mean 0·08, 90% CI 0·01–0·16). In the absence of new infections, the speed of antibody decline was estimated, yielding the predicted antibody concentrations of 22, 7 and 1% of the original at 1, 3 and 10 years, respectively. Without any new exposures, antibody levels would be expected to diminish to about 2 μg/ml after 1 year and to 0·1 μg/ml after 10 years, from the estimated geometric mean value of 10·7 μg/ml for the initial response (c_0).

Antibody concentrations under different forces of infection

Predictions of the resulting antibody concentrations under different forces of infection were then calculated. The effect of subclinical infections on antibody concentrations was visualized by comparing the situation under the estimated mean force of infection ($\lambda = 0{\cdot}25$/year, Fig. 2, middle line) to hypothetical situations with no exposure ($\lambda = 0$/year, Fig. 2, bottom line), or with frequent exposure ($\lambda = 1{\cdot}0$/year, Fig. 2, upper line) during the follow-up. Figure 2 shows that over the long term, the median antibody concentration stabilized to a level regulated by the force of infection. For the force of infection that was actually estimated ($\lambda = 0{\cdot}25$/year), the predicted concentration was 1–2 μg/ml. This figure is consistent with the value observed in the study cohort 3 years after the initial stimulus (geometric mean 1·5 μg/ml), and also with the mean antibody concentration reported for the Finnish adult population at that time [27].

Due to individual variations in the magnitude of antibody response (c_0, 90% CI 8·5–13·3) and in the rate of decline (K), there was heterogeneity in the time the antibody concentration was sustained above a given threshold. Without new encounters with the bacteria ($\lambda = 0$), 90% of individuals would be predicted to have antibodies above 0·15 μg/ml at 1·7 years after the initial antigenic stimulus (subclinical infection or polysaccharide vaccine) and as many as 50% after 8 years. At the same time, some individuals would escape new encounters with the bacteria until their antibody concentrations reached very low values, seen as the long lower tails of the predictive intervals of Figure 2. Under a low force of infection, the infections would be rare, but when they occurred, the individual's antibody concentration would be likely to be low enough ($< 0{\cdot}15$ μg/ml) to permit infection to progress to invasive disease, i.e. every infection would be potentially serious.

If the rate of subclinical infection was as high as one infection per year ($\lambda = 1$/year), the probability of any single individual having an antibody concentration less than 0·15 μg/ml would be very small (Fig. 2). In other words, practically all individuals would receive an antigenic stimulus and therefore mount a new response before the antibody concentration decreased below this level. No invasive disease would be seen in this case.

In practice, only half of the population studied reached the initial concentration of $\geqslant 10\mu$g/ml presumed protective against subclinical infection and in 90% the antibody concentrations had decreased below this level within 2·4 years. Therefore, although the predictions indicated a fairly long protection against invasive Hib disease, protection against nasopharyngeal Hib carriage was seen in a small proportion of the individuals and lasting for a short time period only.

Effect of the force of infection on the number of potentially serious infections

To illustrate the population level interplay between the force of infection and the antibody concentration needed for protective immunity, we have shown in Figure 3 the cumulative number of potentially serious infections (encounters with Hib bacteria at a time when antibody concentration is below 0·15 μg/ml) in two separate, unvaccinated cohorts of 10000 indi-

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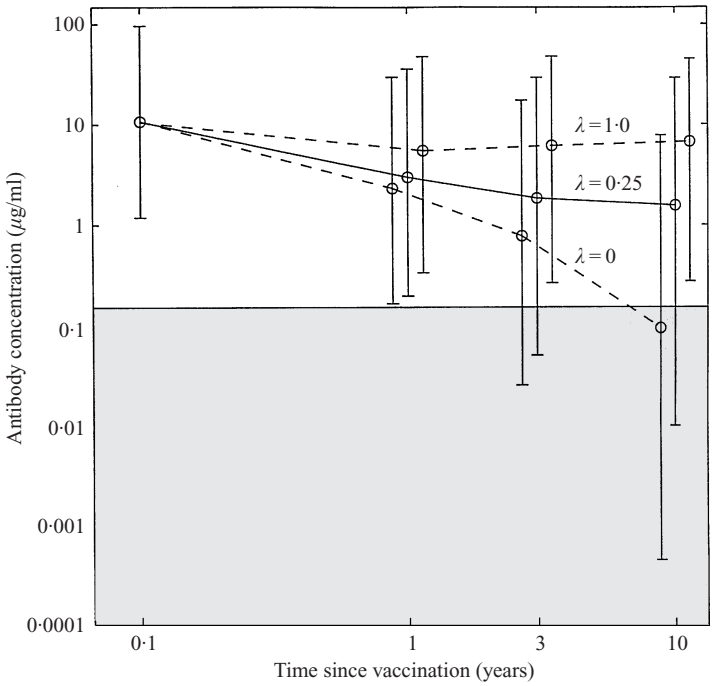

Fig. 2. The median antibody concentrations (circle) and the 90% predictive intervals (bar) over time with three different rates of infection. The uppermost line illustrates the situation under a force of infection $\lambda = 1$/year, the middle line (solid) under $\lambda = 0{\cdot}25$/year, and the bottom line with a zero force of infection. The gray area indicates concentrations below the protective level 0·15 μg/ml.

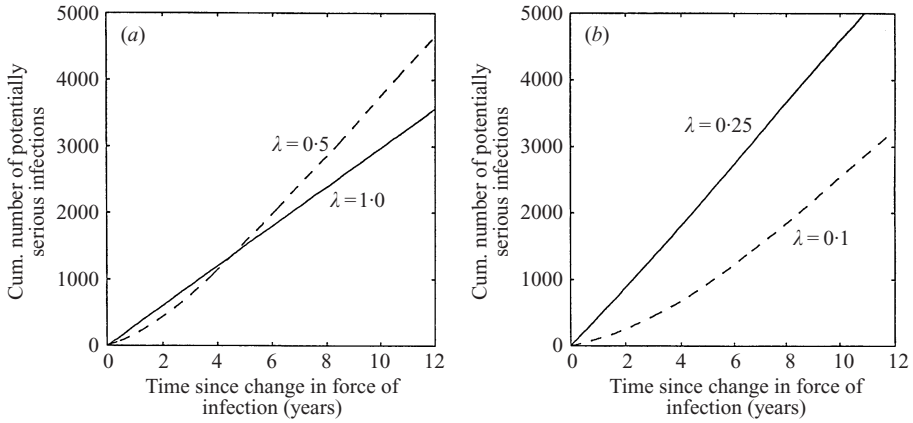

Fig. 3. Cumulative number of potentially serious infections (encounters with Hib bacteria when antibody concentration is less than 0·15 μg/ml) in two cohorts of 10000 unvaccinated individuals. When a part of the population is vaccinated with Hib conjugate vaccine, the force of infection diminishes also among unvaccinated individuals. The predictions for both cohorts are made under two different forces of infection. (*a*) The initial force of infection $\lambda = 1$/year (solid line), the post-vaccination $\lambda = 0{\cdot}5$/year (dashed line). (*b*) The initial $\lambda = 0{\cdot}25$/year (solid line), the post-vaccination $\lambda = 0{\cdot}1$/year (dashed line). Decreasing force of infection may have opposite effects, depending on the initial force of infection. With an initially high force of infection the cumulative incidence of potentially serious infections increases (*a*). With a low initial force of infection the incidence decreases (*b*).

viduals under different forces of infection. Such situations could arise in areas where the force of infection was swiftly reduced by implementation of large-scale vaccinations with Hib conjugate vaccine among children, leaving adults and (depending on the age and coverage of the vaccination) a proportion of children unvaccinated. In Figure 3*a*, the force of infection before the intervention was assumed to be $\lambda = 1$/year (solid line), and after intervention $\lambda = 0{\cdot}5$/year. After few years, this resulted in an increase in number of potentially serious infections in the unvaccinated population, when compared with the

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situation prior to vaccination. Thus, a high force of infection was essential in sustaining protective antibody levels. With a lower force of infection (λ = 0·25/year) as shown in Figure 3*b*, a decrease to λ = 0·1/year would be of advantage even to those not vaccinated, by further decreasing the number of potentially serious infections.

DISCUSSION

In this paper we have presented estimates of the duration of natural immunity to Hib disease under different forces of infection. These estimates, although based on a number of assumptions, can serve as a tool to predict possible changes in the population level of immunity. A new epidemiological situation is arising as conjugated Hib vaccines, providing better protection than natural infections, begin to enter widespread use. Our principal objective was to explore possible changes in patterns of immunity in age cohorts older than 5 years, now widely accepted as being immune to invasive Hib disease. Various infection pressures were used in the calculations to reflect varying Hib epidemiology in different areas (e.g. industrial versus developing countries), consistent with the observed wide variations in incidence and prevalence reported for invasive disease and carriage of Hib worldwide [30]. In each respective situation the force of infection was assumed to be roughly homogenous across the population, allowing comparisons between situations before and after intervention.

We used antibody response to Hib polysaccharide vaccine as a proxy for the response to natural stimulus by subclinical Hib infection. We feel this is justified since the antigen is the same in both cases, and shown to cause a similar T cell independent antibody response [23–26]. We were able to determine accurately the time of antigen exposure and response, and hence the intervals to subsequent measurements (four observations over a period of 3 years). Our statistical model was based on the assumption that the magnitude of the initial antibody response, the rate of decline of antibody concentrations, and the force of infection together determine the antibody concentration at any time after the antigenic stimulus. The model was used to examine, with practical examples, how antibody concentrations can vary in different populations depending on the force of infection and how interventions influence the protective immunity in the population. Analysis of the immune response to polysaccharide antigens is uniquely well suited to this approach, since cell mediated immunity does not need to be considered and all relevant information is thus derived from the measurement of serum antibodies. There are also enough published data to allow a fairly accurate estimate of the antibody concentration protective against invasive disease (0·15 μg/ml, [15–22]).

Duration of immunity to invasive disease

An exponential decline of antibody concentration, as generally assumed when using half-life as its measure, was not consistent with the data in the study cohort. Instead, the analysis showed a markedly slower than exponential decline even though increases in measured antibody concentrations were accounted for by subclinical infections [1]. During the first year the concentration of Hib antibodies was estimated to fall to one quarter of the original, but subsequently it was estimated to take 2 years for the concentration even to halve. The half-life for passively acquired antibodies is of the order of 45 days [21], but the decline in antibody concentration after active immunization (Hib polysaccharide vaccine or natural infection) is clearly different. A decline in antibody concentration after active immunization is a composite of the decay of antibodies in the serum, the decreased production of them, and the death of stimulated B cells producing them, the latter being subject to further regulatory signals [31]. The adequacy of exponential decay as an empirical model for the observed decline of actively produced antibodies has been questioned before, for example in connection with experimental data from studies on hepatitis B antibodies after vaccination [22, 32–35]. Furthermore, earlier models of antibodies to hepatitis B, tetanus, and diphtheria toxoids have shown a clear slowing of the decline with diminishing concentration, i.e. with time since response [36, 37]. This slowing, together with the effect of new stimuli, helps to explain the stability of adult Hib antibody concentration levels, the mean concentration in many populations being around 1–2 μg/ml [15, 27, 38, 39]. Even allowing for some uncertainty regarding the antibody concentration protective for invasive disease, it seems that in the majority of people the duration of protective immunity after active immunization (Hib polysaccharide vaccine or natural infection) is about a decade, even when the force of infection is very low (λ = 0). This finding is consistent both with the rarity of repeated invasive Hib infections, and with the rare

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occurrence of invasive Hib diseases among immunocompetent adults.

Incidence of subclinical infections

The estimated incidence of subclinical Hib infections in the study population was 0·25/year/child. In a recent article, Coen and colleagues derived a lower estimate (0·08, range 0·045–0·123) in fairly comparable populations [40]. The assumptions, as well as technical details in their model differed from ours. They used a population level transmission model to estimate incidence from the observed prevalence of Hib carriage, whereas we estimated incidence from the frequency of antibody responses. The two methods introduce their own sources of error. Carriage prevalences based on culturing nasopharyngeal specimens are likely to be underestimates because of the insensitivity of swabbing and culture, whereas colonization with small numbers of bacteria might be sufficient to stimulate antibody production. Conversely, the incidence estimated in our model is not strictly speaking equal to incidence of Hib but rather to infections stimulating production of Hib specific antibodies. Such infections can be due to Hib or cross-reactive bacteria. Several such bacteria have been identified [11–14] although their role in Hib antibody production is not clear. The closest candidate structurally, *Escherichia coli* K100, has been widely studied but its prevalence is fairly low [41, 42]. The same technical difficulties referred to above in identifying Hib carriage by culture may have led to an overestimation of the role of cross-reactive bacteria when explaining the origin of natural Hib antibodies [3]. For practical purposes, the protective immunity in a population is determined by this broader incidence (both Hib and cross-reactive bacteria taken together) of carriage.

Effect of the force of infection on the number of potentially serious infections

The main goal of Hib prevention at a population level is to minimize the incidence of invasive disease. A major objective of Hib vaccination is therefore to diminish the number of colonizations during which the antibody concentration is below the protective level, generally estimated as approximately 0·15 μg/ml. Polysaccharide vaccination does this by raising the mean antibody levels among those vaccinated. The new conjugate vaccines raise the serum antibody concentration high enough to prevent also acquisition of nasopharyngeal carriage in the vaccinated cohort [4–8] thereby diminishing the force of infection. This in turn would tend to decrease the frequency of subclinical infections in unvaccinated cohorts as well (herd immunity). Indirectly, this would be expected to lead to a lower overall antibody concentration in unvaccinated individuals.

If the force of infection is fairly low to begin with, administration of Hib conjugate vaccine to a part of the population (e.g. young children) and the following decrease of circulating Hib bacteria might result in herd immunity and a decrease in the number of invasive infections in the unvaccinated cohorts as well (Fig. 3b). In areas with a high force of infection, the effect of vaccination on the susceptibility of the population is complicated (Fig. 3a). Following a vaccine intervention, there is a possibility that the incidence of invasive disease in the unvaccinated cohort could increase because of declining antibody concentrations. Unexpected outcomes from interventions with vaccines have been noted and analysed by others [43]. In these cases vaccination has, by diminishing the force of infection, led to an increased mean age of infection for diseases that have more serious complications when contracted in older age (e.g. rubella and measles). In the case of Hib, the mechanism is that protective immunity, formerly possessed by adults, might disappear because the force of infection needed to maintain it, was diminished. The end result could again be a higher incidence of disease in older age groups than seen before the vaccination programme.

Our model suggested that the relation between the rate of decline of antibodies and the force of infection might have a critical role in the population level immunity. A similar interaction has been suggested for malaria [44]. A practical consequence for vaccination programmes using Hib conjugate vaccines is the need to survey during the following decades the incidence of invasive Hib infections in the unvaccinated age groups, older than the classical risk group of the disease. If an increase were to be noted, vaccination of these older cohorts would need to be considered.

However, if bacteria causing production of cross-reactive antibodies have a major role in Hib immunity, protective immunity would not be dependent on Hib contacts alone. In that case, our predictions of post intervention Hib epidemiology presented in Figure 3 would represent the worst case scenarios. In fact, no increase in disease incidence has been observed to date in unvaccinated cohorts in countries that adopted Hib

conjugate vaccination more than a decade ago. This observation is in agreement with the low pre-vaccination force of infection in these countries (Fig. 3*b*). Also it may indeed indicate that cross-reactive bacteria have a role in protective Hib immunity. In the near future, combining insights and results from different mathematical models of Hib epidemiology [1, 40, 45] may permit some quantification of the effect of cross-reactive bacteria on Hib immunity.

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