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Mathematical models of *Haemophilus influenzae* type b

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SUMMARY

A review of empirical studies and the development of a simple theoretical framework are used to explore the relationship between *Haemophilus influenzae* type b (Hib) carriage and disease within populations. The models emphasize the distinction between asymptomatic and symptomatic infection. Maximum likelihood methods are used to estimate parameter values of the models and to evaluate whether models of infection and disease are satisfactory. The low incidence of carriage suggests that persistence of infection is only compatible with the absence of acquired immunity to asymptomatic infection. The slight decline in carriage rates amongst adults is compatible with acquired immunity, but could be a consequence of reduced contacts. The low rate of disease observed in adulthood cannot be explained if protection from disease is a product of previous detectable exposure to Hib alone. We estimate an R_0 of 3·3 for Hib in developed countries, which suggests that current immunization programmes may eliminate the infection. Analysis of the disease data set suggests the absence of maternal immunity and increased susceptibility to disease in the oldest age classes.

INTRODUCTION

The invasion of human tissue by *Haemophilus influenzae* bacteria may result in a variety of clinical conditions ranging from otitis media to pneumonia and meningitis. A proportion of these bacteria possess a polysaccharide capsule. On the basis of their capsular antigens they can be classified into 6 serotypes, a–f [1]. Although all serotypes are capable of causing disease, *Haemophilus influenzae* type b (Hib) is found in more than 90% of invasive disease cases [2, 3]. The introduction of conjugate Hib vaccines was based upon vaccine trials which demonstrated a reduced incidence of disease in vaccine recipients [4, 5]. Since then, the widespread use of vaccine has caused dramatic declines in the incidence of Hib disease [6–9], but many questions about the most efficient vaccination strategies remain, in part because of a limited understanding of the epidemiology of Hib. The development of mathematical models assists the interpretation of epidemiological data through an explicit description of our assumptions about the infection. Here we develop a model of the pattern of carriage and disease caused by Hib.

The infection and the disease

Many bacterial infections, such as those due to Hib and *Neisseria meningitidis* are mostly asymptomatic and may never develop into disease. Such asymptomatic infections are restricted to the upper respiratory tract and are often referred to as 'carriage' or 'colonization'. The prevalence of carriage, or asymptomatic infection, far exceeds the prevalence of disease. Under 5 years of age 26·4 per 100000 children experienced Hib disease in England and Wales every

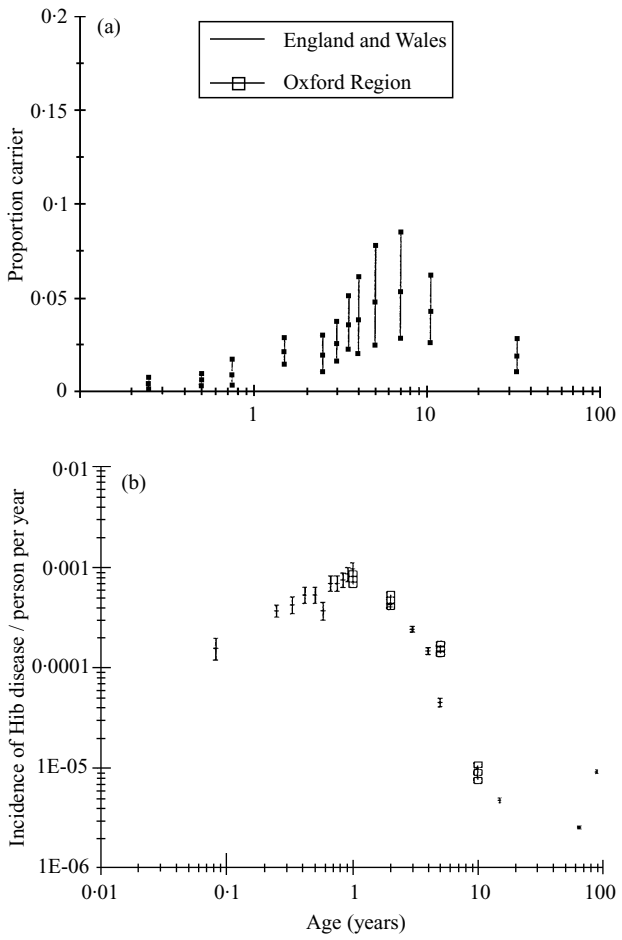

Fig. 1. The prevalence of Hib infection (*a*) and the incidence of incidence of disease (*b*) as a function of age. The data are combined from a number of studies and are used in this analysis to derive parameters. Graph (*a*) represents infection prevalence in Europe and North America between 1970 and 1990 [18, 28, 71–73]. Graph (*b*) represents incidence of Hib disease with 95% confidence intervals in England and Wales between 1990 and 1991 (filled squares) collected by Nazareth and colleagues [10] and incidence of Hib disease the Oxford Region between 1985–91 [47].

year [10] (although there is likely to be under reporting), whereas the prevalence of carriage is approximately 3000 per 100000 [11]. At birth the prevalence of carriage is low, but gradually increases to 3% in early childhood and thereafter declines slightly with age (Fig. 1*a*). By contrast, incidence of Hib disease rises to a peak between 6 and 12 months of age and thereafter declines markedly (Fig. 1*b*), being present but rare in adults [10, 12].

Studies of the incidence of Hib carriage are not as straightforward as studies of the incidence of Hib disease. Invasive disease is easier to identify because of the associated symptoms and the need for hospitalization. Though passive notification of invasive disease can suffer from under reporting, the degree of under reporting of Hib disease cases is moderate and notification trends are generally reliable [13]. Active swabbing of the nasopharyngeal tissues must be undertaken to identify carriage. Swabbing techniques are expensive, time consuming and rely on standardized procedures for the culturing of the isolated organism, giving an estimated congruence of two tests of 83% [14–16, 77]. Oropharyngeal cultures have been shown to be more sensitive than nasopharyngeal cultures [31, 77]. The problems of cost and technical difficulties are exacerbated because the low prevalence of Hib carriage necessitates large sample sizes for accurate estimates. Carriage studies have been undertaken since the 1920s, but serotyping was not introduced until the 1930s and the first studies of serotype b carriage began in the 1940s. However, since then most studies have been restricted to the younger age groups where disease is more common. Studies of carriage in adults have been small and ages were poorly specified.

The importance of carriage in generating immunity to invasive disease is controversial. By analogy with *Neisseria meningitidis*, it is suspected that the first episode of carriage is more likely to cause disease because the immune system has never experienced the infection before. In follow-up studies of US military recruits the acquisition of *N. meningitidis* either led to invasive disease or to the development of protective antibody titres in serum, within a period of 10 days [20]. This confirms the hypothesis that immunity to invasive disease develops during a period of time after the first episode of carriage. Pneumococcal disease also occurs within 1 month of acquisition of a new type of *Streptococcus pneumoniae* [50]. Therefore, by analogy with these infections, there is circumstantial evidence for immunity to invasive Hib disease developing soon after the first acquisition of the organism. It has been hypothesized that the interaction of the bacteria and the mucosal immune system produces natural immunity to disease [21]. Antigens from other organisms, however, can induce cross-reactive antibodies to the Hib capsular polysaccharide, thus the measurement of serum Hib antibody is not as reliable as oropharyngeal swabbing in detecting experience of carriage. Cross-reacting antigens are also capable of 'immunizing' the host [22, 23].

Another controversial issue is whether the diseased case is a more potent transmitter of Hib infection than the asymptomatic carrier. A common observation is that families and day care centres (DCCs) which

experienced cases of disease have greater prevalence of Hib carriage than families and DCCs without such cases [24, 25]. However, it is not clear whether the high carriage prevalences in the other members is responsible for, or a consequence of, the onset of disease in the individual [32]. Therefore the question is unresolved. Nevertheless, it has been established that having pre-school siblings is a risk factor for disease in younger children [26, 27]. This implies that siblings, which are likely to introduce the Hib organism into the household, are responsible for the development of symptoms of the index case, and probably cases of Hib disease do not play a role in the infectious process. This interpretation of the evidence is sensible especially because the index case is hospitalized for most of the duration of illness, and are therefore incapable to transmit infection to 'contacts'.

In contrast, recent studies of carriage among family members of infant vaccinees, it was shown that vaccination of the index case leads to a significant reduction of carriage prevalence among the unvaccinated 3 to 4-year-old siblings compared to controls, a protective efficacy of 48% [28, 29]. Therefore, vaccination must either reduce carriage in vaccinees or transmissibility in infected vaccinees. In either case, this is evidence that infants play a role in the maintenance of carriage within families.

Other upper respiratory tract infections can have a marked effect on the spread of bacterial infection. Carriage of *Haemophilus influenzae* among infants kept in a DCC dramatically increased when a proportion of the children were affected by 'colds' [30, 31]. It is not clear whether cases of invasive disease also cause such an increased transmissibility.

Hib and other bacteria

Populations of *Haemophilus influenzae* bacteria are clonal [33–35]. Multilocus enzyme electrophoresis analysis, which has a sound genetic basis, suggests that serotypes of *H. influenzae* are in strong linkage disequilibrium with each other [33, 37]. This means that genetic traits associated with one serotype will not often switch to another serotype. Because of this genetic isolation and the predominance of Hib in disease incidence, Hib will be modelled as if it were a pathogen whose transmission and serotypic integrity is independent of all other serotypes. It was recently documented that incidence of disease associated with untypable and non-type b serotypes did not change during the Hib immunization era. This is additional evidence that *Haemophilus influenzae* serotypes behave independently of one another [38, 80].

Mathematical models of infection

Mathematics provides a precise language in which assumptions about the epidemiology of an infection can be made explicit. Bacterial infections can be treated in a general framework for micro-parasite infections where the unit of analysis is the state of the host, i.e. susceptible, infected, immune, rather than the intensity of infection [39]. Here we use data on the carriage of bacteria and incidence of disease to explore models of infection and the potential for immunity to play a role in Hib epidemiology.

MATERIALS AND METHODS

A mathematical model of *Haemophilus influenzae*

In developing a model of the dynamics of Hib infection and disease, age structured, deterministic models were compared with reference to observed epidemiological pattern. The full model's compartments are illustrated schematically in Figure 2, where boxes represent the infection and disease categories and arrows represent the flow of individuals between them. All infants are assumed to be born into the susceptible class. Although maternally derived antibodies have been identified [40], as a simplifying assumption their impact was not included. A fuller discussion of this decision is given below. Susceptibles become carriers at a rate λ, the 'force of infection', which is the per susceptible incidence of carriage [39, 41]. Carriers recover at a rate r_1, and a proportion z of these recoveries move to the immune class who are no longer susceptible to the acquisition of carriage. The remainder $(1-z)$ move back to the susceptible class. Carriers can become diseased at an age specific rate, $\phi(a)$, and recover from disease at a rate r_2. Initially carriage was modelled independently of disease, comparing models with immunity (where $z = 1$) and without immunity (where $z = 0$). The parameter z represents the set of models known as SIR (susceptible–infected–resistant) when it is equal to unity, and it represents the SIS (susceptible–infected–susceptible) set of models when it is equal to zero [39]. Given an estimate for the rate of recovery from Hib carriage, an age related data set of the prevalence of Hib carriage was used to estimate age specific forces of infection by means of maximum likelihood [42, 43]. Similarly, an age related data set of

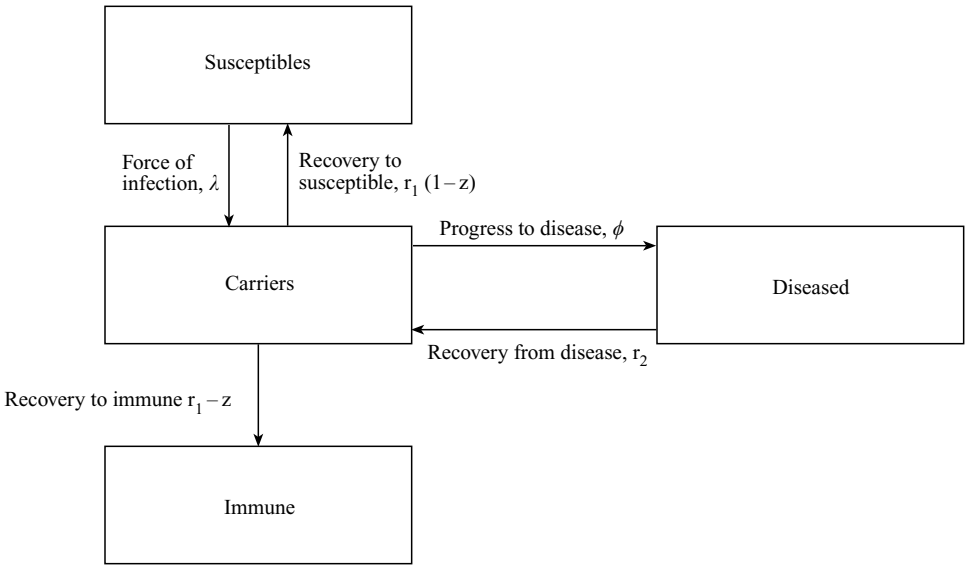

Fig. 2. A schematic representation of the general model of Hib. The z parameter can lead to an SIS model if set to zero, or an SIR model if set to unity. The arrows represent rates of flow of individuals from one compartment to the next. These are the force of infection ($\lambda(a)$), the rate of recovery from infection (r_1), the rate of disease per carrier ($\phi(a)$), and the rate of recovery from disease (r_2).

the incidence of Hib disease was used to estimate age specific rates of progression to disease for carriers, $\phi(a)$.

Model selection technique

A set of differential equations was used to estimate the rates of change of the infectious classes of the model as functions of age. Model expectations were calculated numerically by means of the Runge–Kutta method. The time dimension was dropped from the analysis because the infection is assumed at endemic equilibrium. The goodness of fit of the models was maximized by minimizing the difference between the model log likelihood and the data log likelihood. Twice this difference approximates the χ^2 distribution with $N-p$ degrees of freedom, where N is the number of observations and p is the number of parameters being fitted [44]. Mathematical models were identified which minimized the 'binomial log-likelihood χ^2 deviance function' given by the expression:

$$L = 2. \quad R_a . \ln \frac{R_a}{q_a . N_a} + (N_a - R_a) . \ln \frac{(N_a - R_a)}{N_a(1-q_a)} \quad .$$

When the data set is prevalence of carriage, then q_a is the expected prevalence generated by the model at age a, N_a is the observed number of people sampled within the age class (whose mean age is a), and R_a is the number of carriers found in that age class [45]. The value of L is equivalent to a χ^2 value of the difference between the data and the model. A satisfactory set of models is one where the χ^2 deviation is not significant. Therefore, when fitting models to data our aim is a high probability (P value) for the model to adequately explain variation in the data set. For 95% confidence of 'model truth' a cut-off P value of 0·05 was adopted.

The same method was used for the disease model. However, the disease data set represents incidence, rather than prevalence, of disease over a year, even for single month age classes. To match the data it was necessary to modify the model output. This was done by adjusting the model expectations $D_m(a)$ of the number of Hib cases that occur in an age class of width a, whose oldest age is a, according to the length of the period over which disease incidence was observed, δ. The result is an expression for the adjusted model expectation of cases, D_{adj}:

$$D_{\text{adj}}(a) = D_m(a)\,\delta/a,$$

where $D_{\text{adj}}(a)$ is the value to be compared with the empirical data.

Empirical data on the prevalence of carriage

In order to estimate the force of infection for Hib organisms it is necessary to gather data on the prevalence of infection individuals in the community. The prevalence of Hib carriage is assumed to be a good approximation of the prevalence of infectious

Table 1. *Parameters of the Hib models. Parameter values presented were derived separately whereas those estimated were derived by maximum likelihood from the data sets in Figure 1*

Parameter	Value	Meaning	Source
r_1	2·42	Rate of recovery from carriage, per carrier, per year	[53]
r_2	33·6	Rate of recovery from Hib disease, per Hib case, per year	[36, 47]
λ	To be estimated	Force of infection, per susceptible, per year	Not known
ϕ	To be estimated	Rate of disease, or 'force of disease', per carrier, per year	Not known
z	To be estimated	Proportion of carriers that recover to the recovered class (Fig. 2)	Not known

Table 2. *Data set of Hib carriage. All ages are in years*

Age class	Mean age	Number of carriers	Sample size	Subjects	Source
0·0–0·5	0·25	2	524	Normal	[71]
0·0–1·0	0·50	5	869	Normal	[18, 72]
0·5–1·0	0·75	2	236	Normal	[71]
1·0–2·0	1·50	16	767	Normal	[18, 71]
2·0–3·0	2·50	7	370	Normal	[71]
1·0–4·0	3·00	12	473	Normal	[18, 72]
3·0–4·0	3·50	14	398	Normal	[73]
3·0–5·0	4·00	8	211	Normal	[18]
4·0–6·0	5·00	8	168	Normal	[72]
5·0–8·0	7·00	9	170	Normal	[18]
5·0–16·0	10·50	13	306	Normal	[18]
25·0–40·0	33·50	8	433	Mothers of pre-school children	[55]

individuals. The potential for spread of infection by carriers has never been established with any useful degree of certainty. It is however generally recognized that contact with a carrier is a prerequisite for transmission of the organism [32]. The model used for the estimation of the force of infection assumes that there is no time dependency for carriage prevalence. As there are no known time series studies of prevalence of Hib carriage, the trustworthiness of this assumption remains an open question. The relative stability of the time series of Hib invasive disease suggests that also prevalence of carriage may be at a stable equilibrium in European and North American communities [7, 9].

Empirical estimates of the prevalence of carriage were based on a collection of similar studies (Table 2). To avoid biases, studies of the 'general population' were used as far as possible. Studies from DCCs and disease contacts were therefore excluded from the analysis. To provide reliable measures, sample sizes above 150 swabbed individuals per age class are necessary, and occasionally data for some age classes had to be pooled. To minimize the impact of the changes in incidence attributable to the increased use of day care centres since the 1960s [24, 46], data sets collected before 1970 were not used. We merged data sets from Europe and the United States mainly because the confidence intervals overlapped considerably. It was assumed that the spread of Hib infection within the European community is similar to that for the North American community. The two communi-

Table 3. *Data set of disease collected for England and Wales by Nazareth and colleagues* [*10*]. *The denominators* (*population at risk*) *are from mid year 1993* (*OPCS 1995*)

Age class	Oldest age in class (in years)	Number of Hib cases per year	Population at risk
0–1 month	0·083	3	19645
1–3 months	0·250	14	39290
3–4 months	0·333	8	19645
4–5 months	0·417	10	19645
5–6 months	0·500	10	19645
6–7 months	0·583	7	19645
7–8 months	0·667	13	19645
8–9 months	0·750	13	19645
9–10 months	0·833	14	19645
10–11 months	0·917	16	19645
11–12 months	1·000	18	19645
1–2 years	2·000	92	238743
2–3 years	3·000	56	241275
3–4 years	4·000	33	234581
4–5 years	5·000	10	231923
5–15 years	15·000	10	2208770
15–65 years	65·000	23	9507163
65–90 years	90·000	20	2293861

ties have similar demographic history and are classed together as 'Established Market Economies' [79]. Furthermore, similar swabbing and microbiological techniques were used in studies from both communities [18, 54, 71, 72]. Reliable estimates for the older age classes are problematic because few studies have included them. In the absence of other data a study of Hib carriage in parents of vaccinated and unvaccinated contacts was included [28]. A number of studies show unequivocally that older ages are capable of carrying Hib (Table 4). The combined data sets are presented in Figure 1*a*.

Empirical data for the duration of carriage

A number of studies that attempted to follow-up carriage status in a cohort of individuals have shown that Hib carriers are colonized for at most 6 months, and often for shorter periods [52]. The only study we are aware of with sample sizes sufficient to estimate the duration of carriage by means of maximum likelihood was provided by Michaels and Norden [53]. In this study the prevalence of carriage was followed up from a group of disease contacts. The data set is displayed in Table 3. We used an exponential decay model to estimate the average rate of recovery from Hib carriage from the data set by means of maximum likelihood.

Empirical data on the incidence of disease

The Hib disease data set was derived from the Public Health Laboratory Service (PHLS) Communicable Disease Surveillance Centre (CDSC), which collects information on the incidence of Hib disease from six representative regions that account for approximately 35% of the population of England and Wales [10]. Figure 1*b* compares the CDSC data set with that collected in the Oxford Region by Booy and colleagues [47], and no significant differences are apparent. Table 4 displays the disease data set.

The assumptions of the models

The mathematical models used in this analysis are undoubtedly simplifications of the real world. The approach adopted was to add sophistication to the model only if it were necessary to explain the data sets. For example, as discussed above, Hib transmission is thought to be greater within families whose members have experienced Hib disease [27, 48, 49]. Nevertheless, for modelling purposes, the numbers of diseased individuals were assumed too small to significantly affect the infectious process in the general population. Therefore, the diseased class was excluded from the analysis of the Hib carriage data set. The estimated force of infection was subsequently used

Table 4. *Information available on Hib carriage prevalence in adults. R is the number of carriers identified in the study. N is the sample size. Ages are omitted where information for overall* Haemophilus influenzae *carriage.*

Time period	Location	Age range	R/N	Subjects	Source
1970–4	UK	25–60	6.54	Parents	[49]
1942	US	25–60	1/6	Nurses	[67]
1943–4	US	25–60	4/139	New York employees	[14]
1992–3	UK	25–42	5/158	Mothers: pooled vaccinees and controls	[28]
1990s	UK	25–42	2/131	Mothers	[55]
1973–9	US	25–60	1/151	Adults in day care centres	[24]
1930–7	UK		All adults carried *H. Influenzae*	Employees of the London School of Hygiene and Tropical Medicine	[31]
1925–7	UK		As above	Manchester employees	[65]
1941–6	US		No figures given	Mothers and fathers	[74]
1930	UK		30/30	Nurses	[75]
1958	UK	25–60	2/535	Hospital visitors	[68]
1960	UK		40/252	Bus and train repair workers	[64]
1974	US	25–60	0/160	Adults working in a hospital	[76]
1940–1	UK	25–60	0/60	Adults in an orphanage	[63]

in the estimation of rates of disease per carrier when modelling the disease process. This procedure is made legitimate by the asymmetry in the dependence between the infectious and the disease processes: the latter depends on the former and not vice versa.

Carriage status was assumed to be necessary for the transmission of Hib. It is of course possible that not all infectious contacts result in the development of detectable carriage. If this were true our analysis would make underestimates of the actual force of infection. Obviously there is no evidence for undetectable carriage, and if it exists the models assume they are not important in the infectious process, nor in the immunizing process against Hib disease.

The incidence of Hib mortality was also considered too low to significantly affect the outcome of the models, and it was similarly ignored. The influence of maternally derived protection from carriage was not included, as described above. However, such protection from disease is indirectly included in the models in the form of the estimated age-specific incidence of disease per carrier, $\phi(a)$, along with all other assumptions on the acquisition of natural immunity to Hib disease.

Stochastic simulations

Deterministic approximations are appropriate to explore the behaviour of infection in large populations. However, when dealing with the endemic persistence of low numbers of infections it is necessary to consider stochastic events. Stochastic simulations were used to compare the stability of SIS and SIR models. A simple set of stochastic simulations with exponential waiting times was run as described by Renshaw [51], using a stochastic realization of the same model structure as that used for the deterministic simulations. The parameters used for the simulations

were averages derived from the results of the parameter estimation exercises described above (Table 1), and they are: the rate of loss of carriage ($r_1 = 2{\cdot}42$), the force of infection ($\lambda = 0{\cdot}08$), the life expectancy for the average individual ($al = 75{\cdot}0$), the mortality rate (estimated as the inverse of life expectancy), and the population size ($N = 10000$ and 2000000). Although this is the force of infection estimated for the SIS model this could not be greater for the SIR model or it would result in the exhaustion of susceptibles in the older age classes.

RESULTS

Duration of carriage

We estimate an average duration of carriage of 5 months assuming a constant rate of loss of Hib carriage (Fig. 3). This result is reasonably consistent with those for smaller samples. Amongst children in a Dallas DCC the mean duration amongst six carriers was 2·4 months [52]. It was 6 months in a study of families where disease occurred [49], and Straker and colleagues [31] suggested a mode of 10 months amongst the general population of London for *Haemophilus influenzae*.

Modelling the infectious process

In the analysis of the Hib carriage data sets the SIS model fits the data significantly better than the SIR model ($\chi^2 = 36{\cdot}16$; D.F. = 11, $P = 0{\cdot}00016$; D.F., $P < 0{\cdot}001$; Fig. 4*a*). A functional form for the force of infection suggested by Farrington provided the best fit [43], in comparison to a range of alternative functions, which included the polynomial functions used by Grenfell and Anderson (1995). The form of the function was

$$\lambda(a) = \nu.(\alpha.(a-\gamma).\mathrm{e}^{(-a/\beta)}+\gamma).$$

The estimated values of the four parameters are $\alpha = 0{\cdot}255$, $\beta = 8{\cdot}946$, $\gamma = 0{\cdot}144$, and $\nu = 0{\cdot}122$ ($\chi^2 = 2{\cdot}49$; D.F. = 8; $P = 0{\cdot}96$). The estimated age-specific force of infection is shown in Figure 4*b*. The parameters used in this function are biologically meaningful. The parameter β determines the age at the peak force of infection, α describes the initial rate of increase for the force of infection at birth, and γ describes the value of the force of infection when it reaches a constant value. The last two parameters are discounted by the parameter ν. Based on our analysis we estimate that the peak force of infection occurs at 9 years of age.

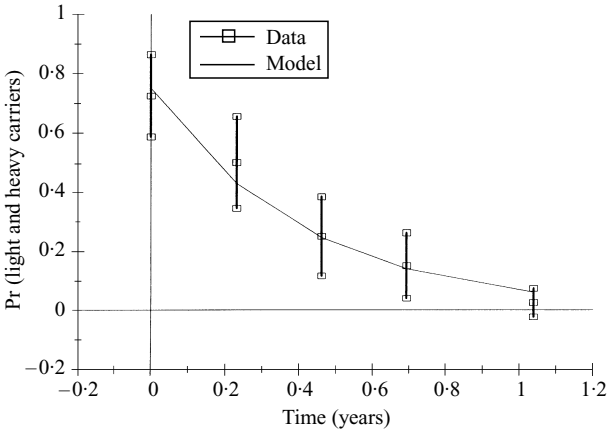

Fig. 3. Proportion of carriers in a cohort of 40 individuals followed up by Michaels and Norden [53]. The study subjects were siblings of children that experienced Hib disease and were recovered at the Children's Hospital of Pittsburgh. The fitted line predicts a loss rate of carriage of 2·42 per year ($\chi^2 = 2{\cdot}12$; D.F. = 4; $P = 0{\cdot}714$).

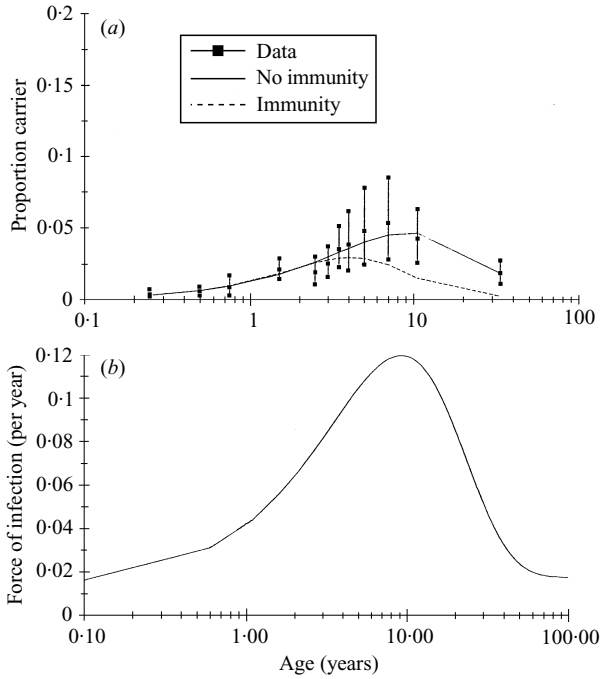

Fig. 4. Results of the parameter estimation analyses carried out for the infectious process. (*a*) Model fit to the carriage data set. The SIS models fitted the data significantly better the SIR models ($P = 0{\cdot}000159$). (*b*) The fitted force of infection as a function of age, $\lambda(a)$. The function suggested by Farrington was one of the best fitting functions [43].

The basic reproductive rate R_0, is a measure of the reproductive capacity of an infection, and it is defined as the number of secondary cases of infection caused in the lifetime of an average infection in an entirely susceptible population [39]. For Hib the situation is more complex because carriers contribute to the

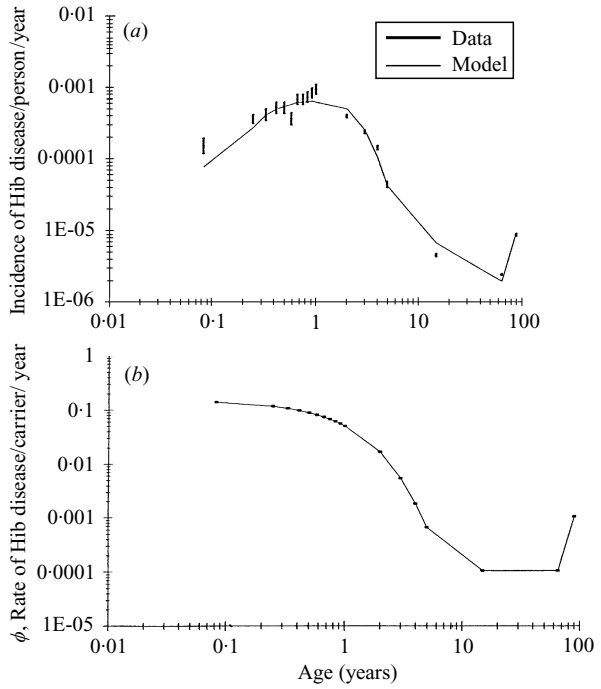

Fig. 5. Results of the parameter estimation analyses carried out for the disease process. (*a*) Model fit to the disease data set. (*b*) The estimated rate of disease per carrier, $\phi(a)$, as a function of age.

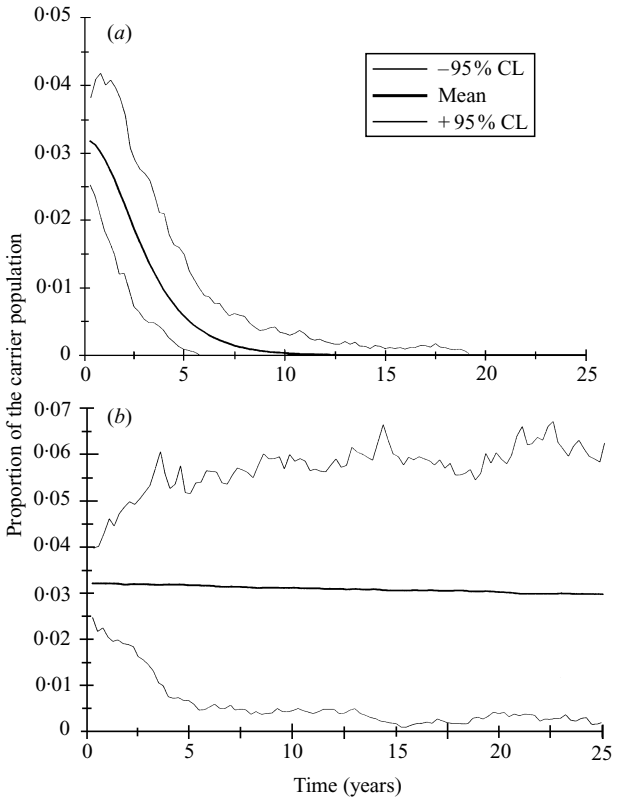

Fig. 6. Prevalence of carriage and time: a comparison between the SIR and SIS models. Each figure shows the minimum and maximum limits for the number of infections at any one time for the core 95% of 1000 stochastic simulations. (*a*) Shows the outcome for the SIR model and (*b*) shows the outcome for the SIR model. The parameters used were: population size, 10000; the force of infection, 0·08; the recovery rate from infection, 2·42. The graphs show the mean (hard line), and 95% confidence limits (broken lines).

infectious process to a much more significant degree than cases of disease. Therefore, the expression 'cases of infection' should translate to 'episodes of carriage'. Thus, the effective reproductive ratio, R_e is the number of secondary episodes of carriage caused by one primary episode in a population with a proportion x of individuals susceptible to the acquisition of Hib carriage. R_e is related to R_0 by the expression,

$$R_e = R_0 x.$$

At endemic equilibrium R_e is expected to be unity because one episode of carriage on average leads to just one more episode. Therefore, the proportion of individuals susceptibles in the population at endemic equilibrium is the inverse of R_0. Using this relationship for the SIS model we estimate an R_0 for Hib of 3·275, with $x = 0{\cdot}305$. Even if the proportion of individuals who have experienced infection is an age dependent variable, Anderson and May [39] show that this is still a valid method for the estimation of R_0, if x is integrated over all age classes.

Modelling the disease process

The estimated rate of disease per carrier was an exponentially declining function of age although the 65–90 years age class appeared to be 10 times more likely to develop disease symptoms than the 15–65 years age class (the model of no increased susceptibility in the elderly: $\chi^2 = 55{\cdot}9$; D.F. = 17; $P = 4{\cdot}9 \times 10^{-6}$). The best fitting function was the exponential one 'corrected' to include a threshold rate of disease γ that represents a lower limit, which is multiplied by the parameter π for ages greater than 65 years:

$$\phi(a) = (a.e^{(-\beta.a)}) + \gamma.\pi \quad (a < 65, \pi = 1)$$
$$(a > 65, \pi > 1)$$

The estimated values for the four parameters are $\alpha = 0{\cdot}151$, $\beta = 1{\cdot}127$, $\gamma = 1{\cdot}035 \times 10^{-4}$, $\pi = 9{\cdot}921$ ($\chi^2 = 18{\cdot}45$; D.F. = 14; $P = 0{\cdot}187$). The fit to the disease data is displayed in Figure 5*a*, and the function of $\phi(a)$ in Figure 5(*b*).

Stochastic simulations

The stochastic simulation runs demonstrate that the SIS models are more stable than the SIR models, where carriers tended to go extinct after a few years of simulation (Fig. 6). All of 1000 SIR simulations resulted in zero infections after 20 years, whereas none of 1000 SIS simulations had lost the carrier class by this time. The same results were obtained for a population size of two million. Intermediate values of z always resulted in the eradication of infection, which suggests that even a small degree of acquired immunity to asymptomatic carriage provides instability to the system.

DISCUSSION

The analysis of the dynamics of *Haemophilus influenzae* type b infection and disease provides a number of insights on the biology of the interaction between Hib bacteria and human hosts. For example, it is possible to make inferences on the nature of immunity to carriage and disease. The SIS model matches empirical data on Hib carriage better than the SIR model, mainly because carriage prevalence in the older age classes is higher than expected with acquired immunity to asymptomatic carriage. The same pattern is observed among related bacterial infections such as overall *Haemophilus influenzae* 11, 39], *Neisseria meningitidis* [81, 82], and *Streptococcus pneumoniae* [unpublished observations]. This result is supported by stochastic simulations which show that prevalence of carriage is more stable under the SIS than SIR models (Fig. 6). In other words the data suggest that human hosts which clear Hib pharyngeal carriage are capable of being re-colonized. The functional form of the estimated force of infection (Fig. 4*b*) also suggest that all ages are capable of acquiring the organism, although there may be a small decline for the older age groups. This decline may be due to behavioural factors, such as a decrease in the frequency of infectious contacts, or it may be due to immunological factors. Unfortunately, the relative importance of behaviour and immunity could not be resolved. The predicted weakness of immunity to carriage of Hib in the human population is not surprising because unconjugated polysaccharide vaccines (PRP), which may mimic the experience of Hib organisms, have also failed to induce protection from carriage in vaccinees [5]. If adults do not develop full immunity against carriage, then maternal immunity to Hib carriage may well not exist in infants, which justifies the absence of the class of maternal immunes from the models. These results contrast with the fact that conjugate vaccines are thought to stimulate the immune system so as to free the vaccinees of Hib carriage [28].

The lack of acquired immunity to asymptomatic carriage suggested here does not preclude the existence of acquired immunity to disease. In fact, it appears that asymptomatic Hib carriage may act as an immunizing process, leading to the development of natural immunity against disease. Hib carriers have been shown to have higher antibody titres than non-carriers regardless of vaccination status [54, 55]. The increased synthesis of DNA and antibody secretion by adenoid organs stimulated by *Haemophilus influenzae* [21] shows that in mucosal tissue Hib might act as a challenge building up protective levels of Hib capsular polysaccharide antibodies in serum.

The models are consistent with the hypothesis that experience of Hib carriage contributes to the development of natural immunity against disease, because the rate of disease per carrier, $\phi(a)$, declines exponentially with age (Fig. 5*b*). If carriage of Hib organisms is a necessary prerequisite for immunity from disease it is reasonable to be wary of interventions aimed at their elimination. If a reduction in carriage prevalence, induced by an immunization programme, raised the susceptibility to disease in the older ages, a perverse epidemiological outcome is possible. In the terms of the mathematical models described here, the disappearance of carriage could result in an increase in the rate of disease per carrier $\phi(a)$ (Fig. 5*b*), should the organism be reintroduced. However, this increase would not be expected if cross-immunity from other organisms plays a significant role in the development of acquired immunity.

The results suggest the force of infection of Hib is in the range between 0·045 and 0·123 per person per year. This is the first study which attempts to estimate forces of infection for Hib in the general population. DCCs, where prevalence of carriage is greater than in the general population, are expected to have greater forces of infection. Murphy and colleagues [52] estimate forces of infection in the range of 0·10–1·55 for a DCC in Dallas.

These results support the theory, presented above, that Hib carriage is not the sole determinant of immunity to disease. A simple argument shows that other factors must play a role if the force of infection for Hib is as low as 0·08 per susceptible per year.

Hence, the proportion of people that have not yet experienced Hib carriage, p(carr), by n years of age is given by

$$p(\text{carr}) = (1-\lambda)^n.$$

After 10 years of age, when most individuals are protected from disease, more than 40% of children will not have experienced Hib carriage. Therefore, experience of Hib carriage could not alone explain the 1000-fold decrease in risk of disease. Undetectable challenges by Hib could explain this discrepancy but there is obviously no evidence for this. In contrast serological studies show antibodies in the majority of children at these ages. By 6 years 95% of children had 'protective' Hib antibodies [22].

Therefore, although the ability of the immune system to protect a first-time carrier of Hib might be expected to improve with age, only the experience of cross-reactive organisms can explain the high prevalence of protective antibody levels in the section of the human population which have not experienced Hib colonization. The existence of organisms that cross-react with Hib capsular polysaccharide has been demonstrated. Bradshaw and colleagues [22] found a range of cross-reactive organisms, that included several strains of *Escherichia coli*, as well as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and group-A β-haemolytic streptococci. Other cross-reactive organisms have also been found in throat cultures [56]. Such organisms are known to be correlated with protection from disease in animal models. For example, *Escherichia coli* organisms of the genotype K100, whose capsular antigens are cross reactive to type b *Haemophilus influenzae* capsular antigen, were found to be protective against Hib bacteraemia and meningitis in weaning infant rats [57]. In comparison with *E. coli* K92 infected controls and uninfected controls Moxon and Anderson [23] demonstrated that *E. coli* K100 infected rats had significantly higher Hib capsular antigen antibody concentrations and lower rates of disease. High serum antibodies to Hib capsular antigen can also be found in rabbits who rarely, if ever, encounter *H. influenzae* type b colonization [22, 58]. However, the role of carriage of K100 *E. coli* in protection from Hib disease is made less clear by the discovery that Hib cases have a higher probability of carrying K100 *E. coli* organisms in their stool samples [59]. Furthermore, even if *E. coli* does play a protective role in the development of natural immunity to Hib disease, this strain is only carried by 2·5% of the human population [56, 59] and is therefore unlikely to be the most important agent of natural immunization against Hib for the human population. It is interesting to note that the Chinese population of Hong Kong has a low incidence of Hib disease, a low prevalence of carriage of Hib organisms, yet have cross-reacting antibodies with the Hib capsular polysaccharides that are neither from Hib organisms or *E. coli* K100 [60].

Hib carriage in the older age classes

The commonly held view that Hib carriage is rare or absent in the adult population is challenged. Although a few studies detected no Hib carriage in adults the results can be explained by their low sample sizes [62, 63]. A greater number of studies provide evidence that adults are capable of carrying *Haemophilus influenzae* organisms [31, 64–66], with a substantial proportion of these being Hib [14, 24, 49, 67, 68]. A literature survey is summarized in Table 5.

The implications of R_0 for the immunization programme

A R_0 as low as 3·275 has practical implications as it can be used to assess the feasibility of immunization programmes in the eradication of infection. Anderson and May [61] introduced a criterion for the assessment of the eradicability for an infection. They use the value of R_0 to estimate a critical threshold proportion of infant vaccinees, p_c, required for the eradication of infection. This proportion is given by the expression

$$p_c = 1 - R_0.$$

The resulting p_c for Hib is low (70%). The result implies that the vaccine used in the current national immunization programme against Hib would not need to generate 100% protection from carriage to accomplish eradication of the organism. Given the high coverages adopted [9], a small degree of protection in those vaccinated or a small reduction in the transmissibility of Hib organisms in vaccinated carriers would be sufficient. Perhaps, as a result of these considerations it is not surprising that mass immunization programmes aimed at Hib infection have resulted in dramatic drops in the incidence of Hib disease [6–9]. To illustrate this property of Hib immunization programmes, Fogarty and colleagues [17] find that 'although more modest vaccine coverage is likely in Ireland (50–75%) a 93% reduction in incidence has occurred in spite of this'. This issue is,

however, made more complex because the post immunization drop in the incidence of Hib disease was expected even without protection from Hib carriage. This is simply because vaccines target infants who are at highest risk of invasive disease. Therefore, a dramatic impact of the vaccines on the incidence of Hib disease was expected for this reason alone. Unfortunately, Hib carriage was not followed up in the post immunization era and the impact of the programme on the proportion of Hib carriers is unknown. Besides, a value of R_0 provides no information on the dynamics of eradication: if it implies that eradication is possible, it does not specify the time required to accomplish it.

As expected, the estimated rate of disease per carrier, $\phi(a)$, is greatest in the younger age classes and then declines towards the older age groups (Fig. 5*b*, [69]). What was unexpected was the finding from this model that the 65–90 years age class appears to have a 10 times higher rate of acquisition of disease than the 15–65 years age class. The finding must be taken with caution, of course, because it depends on a single observation. Nevertheless, the narrow confidence intervals for the disease data set allows us to place some confidence in the result. This observation may be explained by increased debility in the oldest age group. Consistent with this is the recognition that adult invasive disease with Hib occurs in patients with significant underlying conditions including malignancy and alcoholism [12]. Indeed in one study an underlying condition associated with death due to Hib invasive disease was more common in cases younger than 12 months and in those of 65 years of age and older [78]. An immunization programme aimed at the eradication of Hib infection is expected to benefit the older age classes as well as the younger ones.

Furthermore, Figure 5*b* suggests an absence of a protective effect of maternal antibodies of Hib disease in infants. Hib antibodies are transferred from the mother immunized with conjugate vaccine to the baby [70], and infants do have a high prevalence of 'protective' antibody levels [40]. However it is clear that 17 of 126 Hib cases that occurred in children younger than 1 year of age did take place in infants of 0–3 months of age. The low carriage prevalences found in very young infants therefore suggest a significant rate of disease per carrier (ϕ) in the age class prevalent with maternal antibodies. Siber and colleagues [25] did show that human hyperimmune globulin is protective in 2 month old infants. However, natural maternal antibodies may have different properties from artificially transferred hyperimmune globulin.

In summary, models which do not include immunity to carriage provide an adequate description of empirical Hib carriage data. This implies that adults, as well as children, are likely to play an important role in the spread of *Haemophilus influenzae* type b. However, disease incidence decreases as people age, which would be explained by a decreasing rate at which Hib carriers suffer from invasive disease. This could not easily be explained by experience of carriage as an insufficient number of adults has this experience to explain the estimated 1000-fold decrease in progress to disease amongst carriers. Two additional 'immunizing' mechanisms are suggested to explain the discrepancy. One is the improvement in the immune response in first-time carriers of Hib as they age, the other is the possibility that cross-reactive antigens induce natural immunity in the absence of the Hib organism. The relative importance of each of these three mechanisms in the development of protective immunity against Hib disease remains uncertain.

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