Evaluation of the continuing effectiveness of the United Kingdom's Haemophilus influenzae type b national immunisation programme

Thesis

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TITLE PAGE

Name: Dr Jodie McVernon

Degrees: MB BS (Hons), B Med Sc (Hons)

(Monash University, Melbourne, Australia)

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Sponsoring Establishment: Weatherall Institute of Molecular Medicine,

John Radcliffe Hospital, Oxford

Collaborating Establishment: Department of Zoology

University of Oxford, Oxford

Immunisation Department,

Health Protection Agency

Communicable Disease Surveillance Centre,

Colindale, London

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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Abbreviations used in the text

BPIG	Bacterial polysaccharide immune globulin
BPSU	British Paediatric Surveillance Unit
CDC	Centres for Disease Control, Atlanta, GA
CDSC	Communicable Disease Surveillance Centre
CI	Confidence Interval
CMO	Chief Medical Officer
COVER	Coverage of Vaccination Evaluated Rapidly
CSF	Cerebro-spinal fluid
DTaP-Hib	Diphtheria, tetanus, acellular pertussis-Hib combination vaccine
DTwP-Hib	Diphtheria, tetanus, whole cell pertussis-Hib combination vaccine
ELISA	Enzyme-linked immunosorbent assay
ET	Enzyme type
EU-IBIS	European Union Invasive Bacterial Infections Surveillance Network
GMC	Geometric mean concentration
GP	General Practitioner
HbOC	PRP conjugated to CRM_{197}, a mutant diphtheria toxoid
HES	Hospital Episode Statistics
Hi	*Haemophilus influenzae*
Hib	*Haemophilus influenzae* serotype b
HPA	Health Protection Agency
HRU	*Haemophilus* Reference Unit
MEE	Multi-locus enzyme electrophoresis
MLST	Multi-locus sequence typing

ICD	International Statistical Classification of Diseases
MHC	Major histocompatibility complex
MIC	Minimal inhibitory concentration
MMR	Measles, mumps and rubella vaccine
NS-SEC	National Statistics Social and Economic Classification
nt Hi	non-typeable *Haemophilus influenzae*
OMP	Outer membrane protein
ONS	Office for National Statistics
OR	Odds Ratio
OVG	Oxford Vaccine Group
PCR	Polymerase Chain Reaction
PCT	Primary Care Trust
PCV	Proportion of cases vaccinated
PHLS	Public Health Laboratory Service (subsumed into the HPA April 2003)
PPV	Proportion of the population vaccinated
PRP	Poly ribosyl-ribitol phosphate
PRP-D	PRP conjugated to diphtheria toxoid
PRP-OMP	PRP conjugated to *Neisseria meningitiditis* outer membrane proteins
PRP-T	PRP conjugated to tetanus toxoid
RABA	Radioantigen binding assay
SCIEH	Scottish Centre for Infection and Environmental Health
SIRS	Susceptible-infected-resistant-susceptible
SOC2000	Social and Occupational Classification 2000
TD	T cell dependent
TI	T cell independent

TVF	True vaccine failure
UK	United Kingdom
US	United States
V_H	Immunoglobulin heavy chain variable gene region
V_L	Immunoglobulin light chain variable gene region
WAIFW	Who-acquires-infection-from-whom matrix

ABSTRACT

Haemophilus influenzae (Hi) is a gram negative bacillus that commonly colonises the upper respiratory tract of humans. While the serotype b capsular form (Hib) of this organism accounts for less than 5% of carriage episodes, it is responsible for more than 95% of all invasive Hi infections. The capsular polysaccharide of Hib, polyribosylribitol phosphate (PRP), is an important virulence determinant. Serum antibodies specific for PRP correlate with bactericidal activity against the organism, and their acquisition with age in populations has an inverse relationship with the experience of invasive disease.

The first vaccines to be developed against Hib were based on the capsular polysaccharide, but were limited in their application due to the T cell independent nature of the antigen, which was not immunogenic in children less than two years of age. Chemical conjugation of PRP with a carrier protein such as diphtheria or tetanus toxoid converted it into a T cell dependent antigen, capable of eliciting antibody production in infants only a few months old, and resulting in the formation of populations of 'memory' B lymphocytes believed to provide long term protection from infection. Efficacy of these conjugate vaccines against both acquisition of the organism and progression to invasive disease was subsequently demonstrated.

Hib was a significant cause of morbidity and mortality among British children before inclusion of Hib conjugate vaccines in the routine UK infant immunisation schedule in October 1992. In contrast to other developed countries, it was scheduled as a three dose accelerated primary course at 2, 3 and 4 months of age without a booster dose in

the second year of life. Vaccine introduction was accompanied by a catch-up immunisation campaign in which a single dose was given to children under four years of age.

Despite an initial rapid and dramatic fall in Hib disease reports, a resurgence of cases occurred from 1999 onwards, predominantly in vaccinated children. This rise has since been attributed to a number of factors related to vaccine use. Wearing off of the initial catch-up campaign's effect, rapid waning of immunity induced by the accelerated infant schedule and the use of less immunogenic acellular pertussis containing Hib combination vaccines in 2000/2001 have all resulted in a reduction in protective antibody titres in the population at risk. In keeping with the notion that this should correspond with greater likelihood of disease, infants born prematurely, whose response to immunisation is less than term-delivered counterparts, have been shown to have a four fold increase in risk of invasive Hib infection.

Hib vaccines have further been demonstrated to delay acquisition of carriage, thereby reducing transmission in populations. This indirect effect of immunisation, or 'herd immunity' was hailed as a major contribution to disease reduction. It may also impair the maintenance of long term immunity at a population level, however. Following an initial decline in Hib reports associated with vaccine introduction among older unimmunised cohorts, adult case numbers in the UK have now returned to pre-vaccination levels. This rise has occurred in association with a reduction in specific antibody titres to Hib in adult age groups which was observed within only two years of vaccine introduction. In a surprising finding, a national case-control study of invasive Hib infections found a reduced risk of invasive disease among children attending day

nurseries, or exposed to cigarette smoke. Prior to vaccine use, both of these exposures were associated with higher rates of disease, presumed to be mediated through higher carriage prevalence. It is possible that repeated antigenic challenge through colonisation may result in improved persistence of antibodies elicited by immunisation.

The anticipated medium to long term effects of Hib conjugate vaccine as used in the UK on transmission and maintenance of population immunity were simulated using a mathematical model. The model further provided a framework in which to investigate key assumptions regarding the mechanism of vaccine protection. Good correlation of outputs with seroepidemiologic data from the UK was achieved. Exploratory analysis of the protective efficacy of immunologic memory within this system reinforced individual and population level observations of relatively poor protection against invasive disease in individuals whose post-immunisation Hib antibody titres had waned to undetectable levels. The anticipated effects of a range of potential vaccination strategies which may be employed in the UK to raise antibody titres in the age groups at greatest risk and thereby reduce disease incidence were investigated.

The possibility that widespread vaccination may have resulted in an increase in the intrinsic virulence of Hib was considered. No change in the clinical presentation of Hib disease, or its associated mortality, has been observed in immunised or unimmunised children or adults in the UK since vaccine introduction. In the absence of more robust genotypic markers of invasive potential, no evidence exists to suggest that the vaccine has had any significant impact on microbial population structure.

The UK's experience of Hib conjugate vaccines in the medium term has challenged previously held notions regarding the effectiveness of immunologic memory for disease control in populations. Whether this can be ascribed to the limitations of memory per se, or is a particular feature of the immune response to conjugate vaccines, which stimulate T cells irrelevant to the pathogen of interest, is debatable. The importance of long term surveillance for unanticipated effects of immunisation programmes is reinforced. Such considerations are further applicable to the more recently licensed meningococcal and pneumococcal conjugate vaccines.

Chapter 1 – Introduction to the clinical problem of Hib (Literature Review)

1.0 Introduction

Haemophilus influenzae (Hi) is a non-motile gram-negative bacterium found almost exclusively in the upper respiratory tract of humans (Roche RJ et al 1995). Exposure to this family of organisms is common, with estimates of asymptomatic colonisation prevalence ranging from 25-84% in open communities (Moxon ER 1986). Unencapsulated forms of the organism are the most commonly carried, and are a frequent cause of mucosal infections involving the upper and lower airway. In contrast, over 95% of all invasive Hi infections in childhood including meningitis, epiglottitis and bacteraemia, are attributable to the serotype b capsulate form (Hib), which is carried by no more than 5% of the population at risk at any one time (Turk DC 1963) (Mendelman PM et al 1992). Hib meningitis was almost universally fatal before the availability of antibiotic therapy (Ward HK et al 1932). Even with a decrease in mortality, Hib was still recognised to cause between 30 and 50% of all cases of bacterial meningitis worldwide (Peltola H 2000), with high rates of long-term neurological sequelae (Sell HW et al 1972). This ongoing disease burden, together with concerns about development of antibiotic resistance, drove the development of vaccines to prevent Hib infections (Anderson P et al 1985).

In this chapter, we will review the characteristics of the organism, including possible reasons for its particular virulence. In addition, different states of infection leading to the development of natural immunity will be discussed. The application of lessons learned from the study of these host-pathogen interactions to vaccine development, and the way in which they relate to the observed impact of vaccination are explored.

1.1 Initial description of Hib

In her classic paper describing Hi, also known as the 'Pfeiffer bacilli', Pitman described morphologically distinct smooth (S) and rough (R) bacterial colonies growing on Levinthal's transport medium (Pitman M 1931). S organisms were surrounded by discrete capsules and were lethal in animal models at a uniformly lower inoculum dose, thus exhibiting greater virulence than the R colonies. Specific immunoprecipitation reactions were observed when the sera of animals inoculated with an S strain were added to the filtrate of a culture of the same strain, indicating that the S forms were of more than one distinguishable type.

Two serotypes, designated a and b, were differentiated on the basis of these immunological reactions (Pitman M 1931). All of the strains of serotype b (Hib) in this initial study were isolated from cases of meningitis. In later unpublished work, Pitman further characterised four additional S, or capsulate, types c, d, e and f (Turk DC 1963). Spontaneous loss of capsule and reversion of S to R forms was observed *in vitro* after prolonged culture, particularly in the presence of type specific antibody (Pitman M 1931). The R or 'rough' forms are described as non-capsulate or non-typeable (nt Hi).

1.2 Population structure of Hib

More subtle methods for devising the relationships between Hib strains of the same or distinct capsular types have since been developed. Organisms may be characterised on the basis of their biochemical characteristics (biotyping) (Barenkamp SJ et al 1983) (Musser JM et al 1985), or by differences between expressed outer membrane proteins (OMPs) (Musser JM et al 1985) (Martin D et al 1990) and lipopolysaccharide molecules (van Alphen L et al 1983). Examination of the electrophoretic mobility of a range of common

enzymes produced by *Haemophilus* (multilocus enzyme electrophoresis, MEE) was the basis of defining enzyme type (ET) clones, as a means of assessing genetic relatedness (Musser JM et al 1988, 1990). Direct genetic study by restriction fragment length polymorphism (RFLP) analysis of the capsular (*cap)* gene locus (Musser JM et al 1990) or multi-locus sequence typing (MLST) of common housekeeping genes (Meats E et al 2003) has been employed more recently.

These studies have produced a number of interesting observations regarding the global relatedness of Hib strains. Hib isolates arose from one of two separate genetic lineages, both of which were quite distinct from antecedents of other capsular types (Musser JM et al 1990) (Meats E et al 2003). 70% of Hib isolates recovered over a 20-year period worldwide belonged to one of four ET clones (Meats E et al 2003). While a number of Hib clones were widely distributed, only one or two predominated within a given country or large geographical region (Musser JM et al 1990). The distribution of clones appeared most strongly associated with the historical movements of distinct racial or ethnic groups, and showed very little subsequent change or evidence of mixing of strains (Musser JM et al 1990).

Further evidence of lack of genetic transfer between Hib clones was seen in the strong non-random associations that were found when assessing bacterial characteristics by a range of means (Martin D et al 1990) (Musser JM et al 1985, 1988, 1990) (Meats E et al 2003). For example, consistent relationships were found between particular ET clones and OMP types

Figure 1.0 Similarity of phylogenetic relationships between Hi strains, as assessed by MEE and MLST (from Meats E et al 2000)

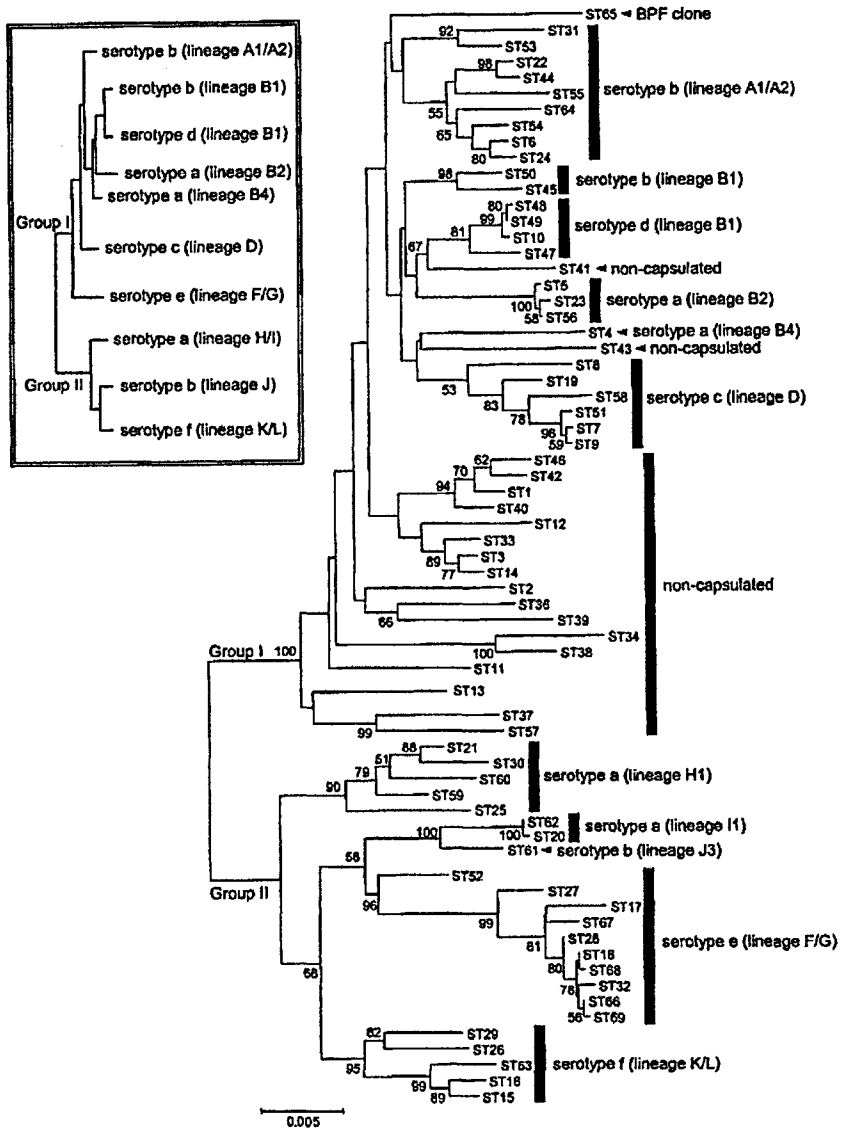


FIG. 3. Phylogenetic relationships among STs of encapsulated and noncapsulated *H. influenzae*. A minimum evolution tree was reconstructed using the concatenated sequences from the seven MLST loci (3,057 bp) for each of the 68 STs. The percent recoveries of the nodes in 1,000 bootstrap replicates are shown where these are ≥50%. The serotypes and MLEE lineages of the STs and the major phylogenetic division of isolates into group I and group II are shown. The tree is rooted at the midpoint of the longest distance between the STs. The box (inset) shows a simplified tree illustrating the relationships between major lineages inferred from the pairwise differences in the electrophoretic profiles of isolates obtained using MLEE. Tree shown in inset adapted from reference 28 with permission of the publisher.

or subtypes. Further, characterisation of the genetic relatedness of capsulate Hi strains by MEE or MLST produced similar results (Figure 1.0). Such features were typical of a highly clonal population structure, suggesting that chromosomal recombination in natural populations was an infrequent event. In particular, it appeared that special barriers existed to the transfer of genes between cap regions (Musser JM et al 1988).

In contrast, far greater diversity of nt Hi strains confirmed that these were not merely capsule deficient serotype b strains, but of distinct genetic lineage with greater evidence of recombination (Meats E et al 2003) (Musser JM et al 1985). Marked diversity in genes encoding lipopolysaccharide was found in nt Hi strains isolated from children with otitis media (Cody AJ et al 2003). This may in part be attributed to greater opportunity for exposure to other organisms, as carriage of multiple genetically distinct isolates of nt Hi within an individual has been well described, while only one clone of a given capsular type of Hi is carried at a time (Trottier S et al 1989) (Smith-Vaughan HC et al 1996) (StSauver J et al 2000).

1.3 Importance of the serotype b capsule as a virulence determinant

Of the *H influenzae* family of organisms, serotype b was noted to be strikingly more common as a cause of invasive disease than the others. Given the distinct genetic lineages from which type b strains derived, it was unclear whether this heightened virulence was directly attributable to capsular expression or other genetic factors. The invasive potential of specific serotypes was examined by inoculating rats with nt Hi strains, genetically transformed to express a range of capsular types (Moxon ER et al 1981, 1984) (Zwahlen A et al 1989). This method ensured that the only phenotypic differences between strains arose from the capsulation locus. Only naturally occurring or transformant serotype b

strains resulted in persistent bacteraemia in these models, confirming the importance of the type b capsular polysaccharide poly ribosyl-ribitol phosphate (PRP) as a virulence determinant.

Serotype b organisms demonstrated enhanced intravascular survival (Moxon ER et al 1981), which appeared to be explained by successful evasion of splenic clearance (Zwahlen A et al 1989). The type b capsule successfully inhibited activation of the alternative pathway of complement, which could be triggered by the organism in the presence of specific antibodies to PRP (Sutton et al 1982) (Steele NP et al 1984). While PRP reduced the rate of opsonophagocytosis of organisms by polymorphonuclear leucocytes *in vitro*, there was no evidence that it did so any more effectively than the capsules of other serotypes a, c, e and f when compared with unencapsulated strains (Crook G et al 1989). The heightened virulence of Hib strains in relation to other Hi capsular types therefore remained imperfectly understood.

1.4 Other factors potentially associated with virulence of Hib strains

There have been reports of particular OMP subtypes of Hib having increased association with invasive disease in outbreak settings (Barenkamp SJ et al 1981) and with clinical presentation with epiglottitis in Finland (Takala AK et al 1987). Such observations have not been subsequently borne out in larger scale analyses. Comparison of outer membrane protein subtypes of isolates derived from asymptomatic carriers or children with invasive infection in population based studies in the United States and Alaska have revealed no significant difference in the frequencies of the most common subtypes between the two sources of strains (Hampton CM et al 1983) (Lucher LA et al 2002). Thus, organisms that

are commonly carried are indistinguishable from those causing serious infections using current molecular techniques.

The ability to modify surface expressed carbohydrates, adhesion molecules and lipopolysaccharide may confer advantage on organisms in adapting to a range of host environments. Different bacterial characteristics facilitate survival in, and transfer between, diverse anatomical sites such as the nasopharynx and bloodstream (Smith AL 1987) (Roche RJ et al 1995). The higher prevalence of carriage of nt Hi would imply improved survival in the nasopharynx without capsule. Some have postulated, however, that capsulation may improve survival outside the host, thereby improving the likelihood of transmission (Moxon ER 1992). Further, Hi enter the bloodstream through being endocytosed by and translocating across human endothelial cells. While capsulation is associated with enhanced survival in the blood, it makes this translocation more difficult (Moxon ER 1992).

Whole genome sequencing of Hib has revealed the presence of a number of hypermutable sites containing simple sequence repeats, known as 'contingency loci' (Bayliss CD et al 2001) that are found within coding or promoter regions. Changes in the number of repeats results in shifting of the reading frame or altered promoter activity, thus acting as an 'on-off' switch within a small number of generations. Phase variable expression of lipopolysaccharide, adhesins and iron acquisition proteins has been demonstrated within Hi (Bayliss CD et al 2001), but microbial genetic factors governing the rate of variation are unknown. In addition, laboratory Hib strains have been observed to vary in the number of copies of the *cap* gene locus, resulting in altered expression of the capsular polysaccharide

PRP and changes in genetic stability (Kroll JS et al 1988). The significance of these findings to survival and virulence *in vivo* requires further study.

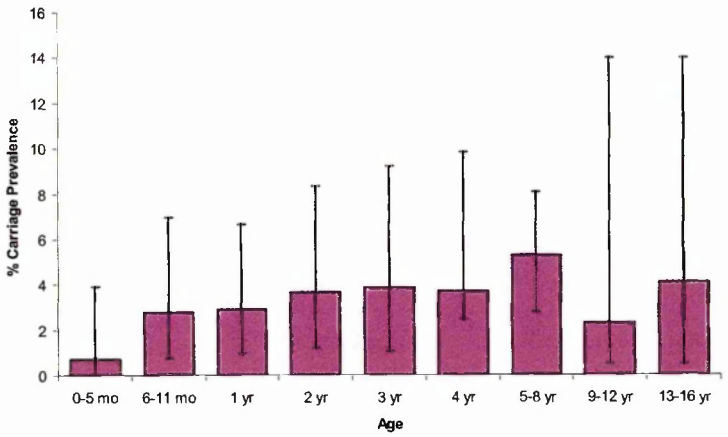
1.5 Asymptomatic colonisation with Hib

H influenzae strains commonly colonise the upper respiratory tract of humans without causing apparent injury. While a broad range of estimates of carriage prevalence have been reported, all studies were agreed that non-typeable forms were approximately ten times more common in asymptomatic individuals than serotype b organisms (Moxon ER 1986). Most estimates of the frequency of Hib carriage have been in the order of 5% or less, but varied according to the microbiological techniques used. Transport of swabs taken from the oropharynx in an enriched broth, followed by plating onto agar containing hyperimmune Hib burro serum, appeared the most sensitive method for detection. Low levels of colonisation, which would be missed by plating onto chocolate agar in the first instance, could be identified by these means (Michaels RH et al 1975).

A population based US study conducted in the 1970s utilising these sensitive microbiological methods reported carriage rates of 0.7% in infants under 6 months, rising to between 3 and 5% throughout older childhood and adolescence (Michaels RH et al 1976) (Figure 1.1a). Fewer carriers were identified in a Welsh cross sectional survey of children up to six years of age, with Hib isolated from only 1.1% of 996 subjects (Howard AJ et al 1988) (Figure 1.1b). In the latter study however, swabs were taken from the nasopharynx and inoculated directly onto chocolate agar plates, making comparison of these findings in the two populations difficult. The figure of 1% was very similar to that obtained in a

Figure 1.1: Cross sectional population based studies of Hib carriage prevalence (error bars represent 95% confidence intervals)

a) United States, data from Michaels RH et al 1976



b) Wales, data from Howard AJ et al 1988

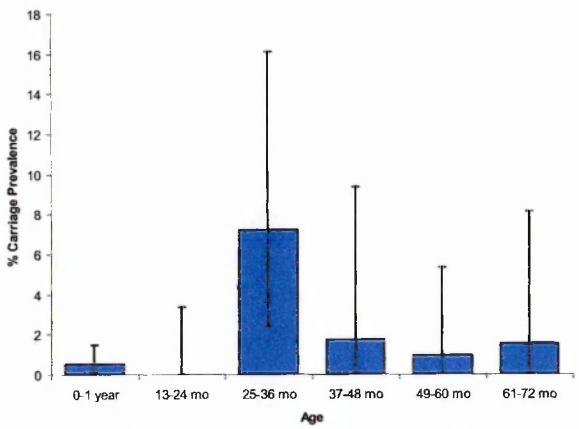
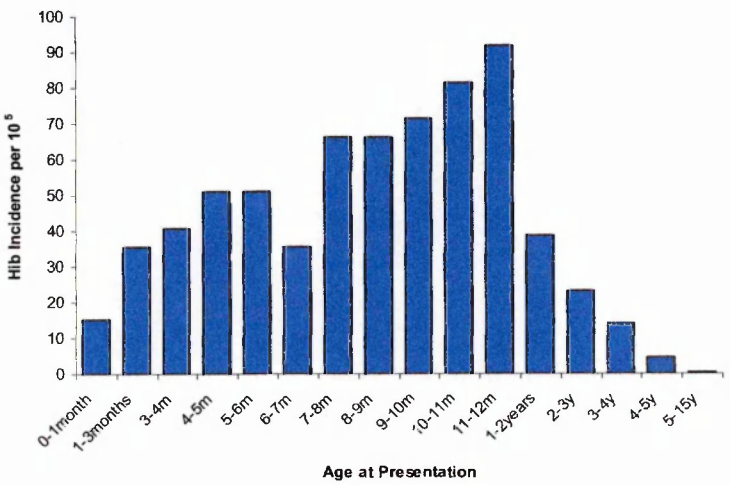


Figure 1.2: Age specific incidence of invasive Hib infection, England and Wales 1990-1992, data from Anderson EC et al 1995



survey in Leeds in the 1950s using the same direct plating methods, where 6 of 650 children under 5 years were found to carry serotype b strains (Dawson B et al 1952).

Several other features of asymptomatic Hib infection have been characterised. A time lag of several weeks was observed before acquisition of Hib strains in infants cared for in a single room in an orphanage in Jamaica. This delay suggested that Hib transmission did not occur readily (Turk DC 1963). Similarly, among household contacts of children recovered from invasive Hib infection followed over one year, more than half of the initially uncolonized siblings remained so for six months or more while constantly exposed to family carriers (Michaels RH et al 1977). Close and prolonged contact between individuals would therefore appear to be necessary for spread of the organism to occur.

In a longitudinal study of carriage in nursery attendees, a wide range of values for the duration of colonisation was observed, with an average estimate of 2.4 months (Murphy TV et al 1985). Observation of infants repeatedly cultured in a Jamaican nursery similarly suggested that clearance of Hib usually occurred within 2-3 months, although not all children could be followed for prolonged periods (Turk DC 1963). In the Michaels and Norden family study described above, some individuals carried for up to one year, although this figure may represent successive episodes of clearance and reacquisition (Michaels RH et al 1977).

One British longitudinal study suggested a seasonal pattern to Hib carriage, with more carriers detected in the autumn and winter months than in summertime (Heath PT et al 1997). It was difficult, however, to distinguish between a true impact of climate and temporal variation in mixing between new groups of children, as occurs with

commencement of school or nursery attendance. Among 66 children repeatedly swabbed in a nursery over an 18-month period, the highest rates of colonisation were detected in the first six months of the survey (Murphy TV et al 1985). This may be postulated to have been due to introduction of naïve individuals into the group, who became colonised for the first time, rather than environmental factors.

1.6 Risk factors for Hib carriage

As seen in the population surveys described above (Michaels RH et al 1976) (Howard AJ et al 1988), carriage of Hib before the age of 2 years was relatively uncommon (Principi N et al 1999) (StSauver J et al 2000). In the Welsh carriage study, the only children under 2 who were carriers were those with older siblings living in the household (Howard AJ et al 1988). The role of an older child with wider social networks as the introducer of Hib to the family was confirmed in subsequent case-control studies (Principi N et al 1999). Further, the number of people living in the home had a direct relationship with the likelihood of carriage, but this association lost its influence once children began attending school on a regular basis (Lerman SJ et al 1979). The importance of mixing with children outside a normal family setting to acquisition of Hib was also shown by the higher prevalence of colonisation demonstrated in children in orphanages (Turk DC 1963) (Mpairwe Y 1970) and attending day care centres (Turk DC 1963) (Murphy TV et al 1985) (Stephenson WP et al 1985) (Principi N et al 1999).

Close contacts of a child with invasive Hib infection exhibited markedly increased rates of carriage compared with those expected in the wider population. This association was observed in household (Johnson RD et al 1943) (Boisvert PL 1948) (Turk DC 1963) (Michaels RH et al 1975)(Michaels RH et al 1977) (Ward JI et al 1979), nursery (Ginsburg

CM et al 1977) (Prober CG et al 1982) (Murphy TV et al 1983a) (Li KI et al 1986) (vonRosen IA et al 1990) and institutional (Johnson RD et al 1943) (Straker EA 1945) (Glode MP et al 1976) settings. Similarly, secondary household contacts of children exposed to a case exhibited higher colonisation prevalence than would otherwise have been expected (Murphy TV et al 1983a) (Li KI et al 1986). Causality in this association remains unclear: are Hib disease cases more likely to arise in situations where carriage prevalence is already high, or are there features of the organisms causing serious infections, or of the disease state itself, that contribute to heightened transmissibility of strains? By definition, invasive Hib infections involve transfer of the organism to occult sites such as the bloodstream or cerebrospinal fluid (CSF), from where spread to other hosts is unlikely to occur. Very high rates of carriage have been repeatedly observed without disease (Turk DC et al 1963) (Murphy TV et al 1985). The possibility that characteristics of specific strains may make them both more transmissible and more invasive remains intriguing.

Integrity of mucosal surfaces may be important in reducing the likelihood of bacterial adherence. Factors associated with damage to the mucosa, including exposure to cigarette smoke and a history of atopy have been linked with carriage of multiple strains of nt Hi in one study (StSauver J et al 2000).

1.7 Invasive disease due to Hib

Despite a population prevalence of Hib carriage in the order of 1 to 5% among children in the developed world, serious infections due to this organism were relatively rare, affecting between 30 and 60 per 100,000 children within the first five years of life (Clements DA et al 1993) (Peltola H 2000). In addition, the relationship between carriage prevalence and invasive disease incidence was not consistent with age. While Hib colonisation in

population surveys was uncommon in children less than 2 years old (Michaels RH et al 1976) (Howard AJ et al 1988), the vast majority of paediatric Hib disease cases in industrialised countries occurred between 6 and 24 months of age. The incidence then fell sharply, becoming rare after the 5th birthday (Fothergill LD et al 1933) (Booy R et al 1993) (Anderson EC et al 1995) (Figure 1.2). The proportion of all paediatric disease experienced in the first 12 months of life was much greater in the developing world (Peltola H 2000). This trend to early infections was also marked among indigenous populations in Alaska (Ward JI et al 1981), northern Canada, the United States (US) (Siber GR et al 1990) (Wolff MC et al 1999) and central and northern Australia (Guthridge S et al 2000).

Common clinical presentations of invasive Hib disease included meningitis, epiglottitis, cellulitis, bone and joint infections, bacteraemia and pneumonia (Booy R et al 1993) (Anderson EC et al 1995). Globally, 52% of bacteraemic Hib infections presented with meningitis. Hib was further estimated to be the causative organism in between 30% and 50% of all cases of childhood bacterial meningitis (Peltola H 2000). Hib was almost the sole cause of epiglottitis in children (Berenberg W et al 1958) (Gorelick MH et al 1994) (Hugosson S et al 1994). The incidence of epiglottitis varied between developed countries for reasons that were incompletely understood, with the highest rates observed in Switzerland and Australia (Clements DA et al 1993) (Peltola H 2000). The importance of Hib as a cause of non-bacteraemic radiologically defined pneumonia in the developing world was highlighted by a 20 to 25% reduction in this disease presentation in children immunised against Hib in a large phase III vaccine efficacy study conducted in the Gambia (Mulholland K et al 1997). This recent finding led to substantial re-estimates of the global disease burden of Hib (Peltola H 2000).

Prior to the availability of effective antimicrobial therapy, Hib meningitis in infancy was usually fatal, with an estimated mortality in the US in the 1930s of 92% (Ward HK et al 1932). Early attempts at intrathecal therapy using hyperimmune horse serum resulted in some reduction in bacterial count within the CSF of patients with Hib meningitis, but unfortunately relapse and death occurred in all but one of 13 cases (Pitman M 1933). Administration of antiserum with complement fared only slightly better (Ward HK et al 1932). With the advent of antibiotics including penicillin and streptomycin, chances of survival improved considerably (Braid F et al 1949). In many parts of the developing world, case fatality rates associated with Hib infection remain high, largely due to limited access to medical resources (Ferreccio C et al 1990) (Bijlmer HA et al 1992) (Steinhoff MC 1998). In the US and Europe, less than 5% of all Hib infections in the antibiotic era result in death (Clements DA et al 1993), with the highest risk of mortality in neonates and the elderly (Anderson EC et al 1995).

In spite of the improved survival associated with antibiotic use, long-term neurological morbidity after recovery from Hib meningitis was common. Sequelae included hearing impairment and global developmental delay, with estimates of severe residual disability occurring after 4-30% of cases, and moderate sequelae in 5-14%, depending on the methods of assessment and definitions employed (Sell HW et al 1972) (Feigin RD et al 1976) (Clements DA et al 1993). Thus, prevention rather than treatment of Hib infections remained a desirable objective.

1.8 Risk factors for invasive Hib disease

Social and environmental risk factors for invasive disease due to Hib were very similar to those associated with increased carriage rates. As early as the 1950s, it was noted that Hib

cases were more likely to have an older sibling at home than the rest of the population (Ounsted C 1950) (Ounsted C 1951) (Turk DC 1975) (Istre et al 1985) (Zielinski A et al 2003). Again, a dose dependent relationship with the number of exposures to other family members was observed, with increasing household crowding conferring additional risk (Cochi SL et al 1986) (Clements DA et al 1992) (Vadheim CM et al 1992).

In a series of case-control studies, enrolment in day care nurseries (Istre et al 1985) (Cochi SL et al 1986) (Takala AK et al 1989) (Clements DA et al 1992) (Fogarty J et al 1995) (Zielinski A et al 2003) was the most consistent single variable overrepresented among cases. The age specific relative risk of invasive Hib infection in day care decreased with age from 12.3 for children less than one year, to 7.2 in 1-2 year olds and 3.8 in 2-3 year olds in one large epidemiologic study (Redmond SR et al 1984). Not surprisingly, the impact of day care attendance was greatest for children without older siblings in the household (Takala AK et al 1989) (Clements DA et al 1992).

Low parental education (Broome CV 1987a) and low household income (Vadheim CM et al 1992) were identified as risk factors for Hib disease in two studies. The extent to which the relationship with these socio-economic indices was mediated through other variables was not clear.

Exposures having an impact on mucosal integrity and immunity were associated with alterations in risk. The presence of smokers in the household increased the likelihood of disease in one study (Vadheim CM et al 1992). Breast-feeding was protective in several studies, particularly in the first 12 months of life (Cochi SL et al 1986) (Takala AK et al 1989) (Petersen GM et al 1991) (Silfverdal SA et al 1997) (Wolff MC et al 1999).

Close contact with a child with Hib disease was a strong risk factor for carriage, as noted above. Similarly, secondary cases of invasive Hib infection were rare but well described in household (Filice GA et al 1978) (Ward JI et al 1979) (Trollfors B 1991) and day care settings (Fleming DW et al 1985) (Osterholm MT et al 1987) (Murphy TV et al 1987) (Makintubee S et al 1987) (Marks MI 1987). Rarely, a child recovered from Hib infection experienced a recurrence of disease, arising either from persistent Hib carriage (Edmonson MB et al 1982) or reinfection from a close contact (Cates KL et al 1987). On these grounds, Rifampicin was recommended for children recovering from Hib disease and their household members in order to break the cycle of transmission (Brunell PA et al 1984) (Cartwright KAV et al 1991). Chemoprophylaxis was also advocated for infants in a day care centre where two or more cases of invasive disease had occurred within 60 days in the US (Brunell PA et al 1984) or within 120 days in Britain (Cartwright KAV et al 1991). When given at a dose of 20 mg/kg/day for at least 2 days it was shown to be safe and efficacious in eliminating Hib carriage (Murphy TV et al 1983b) (Glode MP et al 1985) (Campos J et al 1987). Prevention of secondary disease cases was demonstrated when Rifampicin therapy was compared with placebo in randomised controlled trials (Band JD et al 1984).

A number of underlying medical conditions were identified as conferring increased risk of Hib disease in several studies. Particular host factors predisposing to infections with encapsulated organisms included sickle cell disease (Goldblatt D et al 1996), immunodeficiency syndromes (Farrand RJ 1970), a history of recurrent infections (Takala AK et al 1989) (Silfverdal SA et al 1997) and some malignancies (Broome CV 1987a). A

preceding history of non-specific chronic illness (Vadheim CM et al 1992) (Fogarty J et al 1995) was also over-represented amongst cases.

In the US, African-Americans experienced a higher rate of Hib infections than the Caucasian population, although the extent to which this resulted from confounding due to social factors was not clear (Broome CV 1987a) (Vadheim CM et al 1992). Among indigenous populations in North America and Australia the annual incidence of Hib meningitis alone exceeded 150 per 100,000 in children under 4 years of age (Peltola H 2000). High transmission pressure was believed to contribute to increased rates and early onset of disease in these communities (Ward JI et al 1981). Immunoglobulin subclass deficiency was noted in Apache children and suggested as a further aetiologic factor (Siber GR et al 1990). Genetic differences in immune responsiveness to Hib capsular polysaccharide have also been implicated in the marked excess of disease observed in these ethnic groups (Siber GR et al 1990) (Lucas AH et al 1999) (Guthridge S et al 2000).

1.9 Development of natural immunity against Hib

In 1932, Ward and Wright observed that the bactericidal power of blood against 'influenza bacilli' depended primarily on humoral antibody (Ward HK et al 1932). Fothergill and Wright extended these studies in order to seek an explanation for the striking age specific incidence of invasive Hib infections (Fothergill LD 1933). The bactericidal activity of human sera collected across a wide age range was tested against a fatal 'smooth' strain of Hi isolated from a case of meningitis. A reciprocal relationship between bactericidal activity and invasive disease incidence was demonstrated, with maternally derived antibody providing initial protection, waning to leave infants susceptible over the first few years of life. From the age of 3 onwards, levels of immunity were observed to rise, becoming permanent in adulthood (Fothergill LD 1933) (Figure 1.3a). These findings were later repeated in similar studies (Norden CW 1974).

Alexander and co-workers provided conclusive proof that the bactericidal antibodies in hyperimmune serum were specific for Hib capsular carbohydrates, by demonstrating loss of functional activity of serum after absorption with a purified capsular polysaccharide preparation (Alexander HE et al 1944). Strong correlation between antibody directed towards PRP and the ability to kill Hib strains *in vitro* (Anderson P et al 1972) (Johnston RB et al 1973) and *in vivo* in animal models (Shaw S et al 1976) (Schneerson R et al 1971) was subsequently demonstrated.

More recent population studies of the age distribution of prevalence of anti-PRP antibodies consistently found the same inverse relationship with disease incidence as that observed by Fothergill and Wright, whether measuring their concentration by haemagglutination

Figure 1.3: Relationship between humoral immunity against Hib and invasive disease incidence, by age. Figures from:

a) Fothergill LD and Wright J 1933

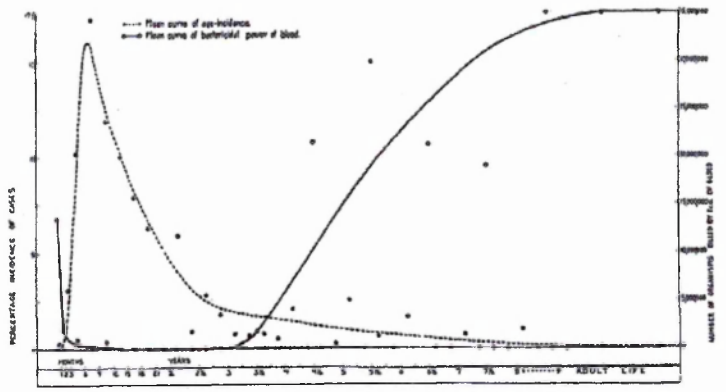


CHART 3. THE RELATION OF AGE INCIDENCE OF INFLUENZAL MENINGITIS TO THE BACTERICIDAL POWER OF HUMAN BLOOD AT DIFFERENT AGES AGAINST A SMOOTH MENINGEAL STRAIN OF B. INFLUENZAE

b) Anderson P et al 1977

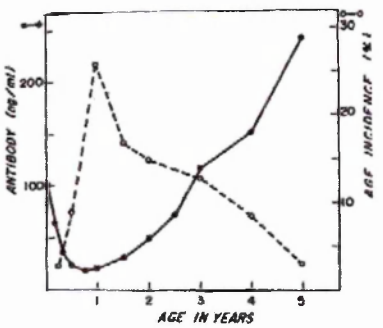
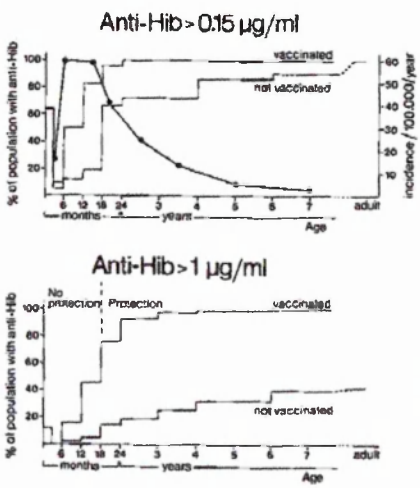


Figure 1. Age distribution of naturally acquired antibody to *Haemophilus influenzae* type b in healthy children and of meningitis due to *H. influenzae* type b. The antibody data are the geometric mean titers (table 2), and the incidence data are the percentages of 397 patients admitted to the Children's Hospital Medical Center (Boston, Mass.) during the years 1961-1973 [12].

c) Kayhty H et al 1983



Legend. Incidence of meningitis due to *Haemophilus influenzae* type b (Hib) (*top*; ●——●) and protective effect of the Hib capsular polysaccharide vaccine (*bottom*) compared with the percentage of the population at each specified age having levels of antibody to Hib polysaccharide (anti-Hib) exceeding 0.15 µg/ml (*top*) or 1 µg/ml (*bottom*) before and after immunization with the Hib vaccine. Levels of anti-Hib were measured by radioimmunoassay using as the antigen a ^{125}I-labeled tyramine derivative of the Hib polysaccharide, and as the reference the standard serum "SK" [1], obtained from Dr. John Robbins (Bureau of Biologics, Food and Drug Administration, Bethesda, Md.). Serum samples were taken from 514 children between the ages of three and 96 months before and three weeks after immunization with a single dose (12.7 µg) of Hib vaccine, as well as from unimmunized adults and neonates. The incidence of meningitis due to Hib was calculated from reports covering the six-year period from 1975 to 1981 in Finland. The protective efficacy of the Hib vaccine was taken from a previous report [2].

(Schneerson R et al 1971), radioantigen binding assay (RABA) (Anderson P et al 1977a) (Kayhty H et al 1983a) (Sansoni A et al 1992) (Hazlewood M et al 1993) or enzyme linked immunosorbent assay (ELISA) (Ballereau F et al 1999) (Trotter CL et al 2003) (Figure 1.3b). A naturally occurring antibody concentration of 0.15 μg/ml showed the best reciprocal correlation with meningitis incidence in one study in Finland (Kayhty H et al 1983a) (Figure 1.3c). On this basis, an antibody threshold of 0.15 μg/ml was defined as 'protective' in the short term against invasive Hib infections (Kayhty et al 1983a).

Rising anticapsular antibody titres within individuals have been temporally associated with known exposure to Hib following documented episodes of carriage (Michaels RH et al 1977) (Hall DB et al 1987) and recovery from invasive Hib infection (Schneerson R et al 1971) (Kayhty H et al 1981) (Trollfors B et al 1992). Circumstantial evidence of asymptomatic infection in close contacts was inferred from evidence of boosting of specific immunity to Hib in children and adults exposed to a disease case in their household (Norden CW 1974) or nursery (Greenfield S et al 1972). The magnitude of the antibody response increased with age, and was greater in children recovering from epiglottitis than meningitis (Johnson PDR et al 1996). Of note, hyporesponsiveness to the Hib capsular polysaccharide was observed in children aged less than 18-24 months, who exhibited minimal or no serum antibody response to either exposure to the organism or immunisation with purified PRP (Norden CW et al 1972) (Anderson P et al 1977a) (Michaels RH et al 1977) (Kayhty H et al 1981). In contrast, the production of specific anticapsular mucosal IgA during convalescence from invasive Hib disease appeared to be independent of both age and serum IgG levels, and was observed in even the youngest infants (Pichichero ME et al 1981).

Poor serum responses to polysaccharides in human infants are in keeping with a progressive sequence of immune responsiveness to different forms of antigen seen in humans and other higher vertebrates. Proteins are consistently recognised earlier in ontogeny than carbohydrates (South MA 1972) (Howard JG et al 1976) (Goldblatt D 1998a). Capsular polysaccharides are able to directly stimulate mature B lymphocytes to produce antibody without the assistance of T cells, and are thus described as 'T cell independent' (TI). Immature B cells are unable to respond to TI antigens without the extra stimulation afforded by T cell help (Goldblatt D 1998b) (Rijkers GT et al 1998) (Valiante NM et al 1999). This observation has been attributed to general immaturity of the B cell compartment in infants (Nossal GJV 1997), which may be due to diminished expression of CD21 on the surface of neonatal B cells. CD21 is a co-stimulatory receptor that binds activated complement components (Rijkers GT et al 1998). An alternative view suggests that hyporesponsiveness may simply reflect lack of previous priming exposure to polysaccharides in the context of a whole organism, and so is more a marker of inexperience than incapacity (Hougs L et al 1999). Whatever the cause, this inability to generate a specific immune response against Hib in the first two years of life can be seen to correlate with the highest incidence of invasive disease (Figure 1.3a-c).

Antibodies to a range of surface exposed OMPs and lipopolysaccharide were also demonstrated in the serum of children convalescing from Hib infection (Gulig PA et al 1982) (Claesson BA et al 1987) (Johnson PDR et al 1993). This observation stimulated enthusiasm regarding the potential role of such antigens as vaccine candidates (Gulig PA et al 1982). Early reports of passive protection induced by OMP antibodies in an infant rat model (Shenep JL et al 1983) were not confirmed in subsequent studies, however

(Srikumar R et al 1992). This line of enquiry into vaccine development was therefore terminated.

A range of other organisms including E coli K100 and an Actinobacillus species express cell wall antigens that cross-react with the Hib capsular polysaccharide (Robbins JB et al 1973) (Myerowitz RL et al 1978) (Insel RA et al 1982). Of particular note, infants may be able to mount an immune response to such antigens at an age when hyporesponsiveness to PRP persists (Robbins JB et al 1973). As such, they may have an important role in priming for natural immunity. These 'cross reactive' antibodies were demonstrated to exhibit immunochemical and bactericidal activity against Hib (Myerowitz et al 1978). While cross reactive antibodies consistently bound Hib capsular polysaccharide, however, the binding of antibodies induced by Hib infection or PRP immunisation to radiolabelled PRP was not inhibited by addition of free E coli K100 capsular polysaccharide (Insel RA 1982). Further, vaccination with PRP reduced the proportion of antibodies that were cross reactive in post-immunisation sera compared with pre-immunisation sera (Insel RA et al 1982). Thus, focussing of the immune response towards PRP may be expected to occur over time as antibody specificity is skewed in favour of the Hib capsular polysaccharide.

1.10 Function of anticapsular antibodies

Antibodies to PRP activate both the classical and alternative complement pathways (Steele NP et al 1984), and opsonise the organism for phagocytosis (Musher D et al 1986). Variable correlation between serum IgG levels and bactericidal function has been observed within individuals (Musher D et al 1986). Such diversity can be explained by differences in the avidity of antibody produced in response to PRP exposure (Amir J et al 1990) (Nahm MH et al 1995). Avidity refers to the strength of binding of antibodies to long

polysaccharide chains (Nahm MH et al 1995). Antibodies with weaker binding exhibit less function in *in vitro* assays. TI antigens elicit short-lived antibody responses that do not demonstrate classical maturation of avidity, but clonal selection of B cells may result in a slight drift towards increasing avidity over time within an individual (Goldblatt D 1998a).

Very high serum levels of specific IgG antibody prevented adherence of Hib to the nasal mucosa of infant rats (Kauppi M et al 1993) (vanAlphen L et al 1996). At lower titres, bacterial replication at the mucosal surface was diminished, thereby reducing colonisation density without eradication (Kauppi M et al 1993) (van Alphen L et al 1996). These actions of anti-PRP antibodies to prevent or impair colonisation were not mediated through the Fc component of the antibody molecule, and were therefore not dependent on complement activation or phagocytosis (Kauppi-Korkeila M et al 1996). The exact mechanism of interference with carriage remains unclear, as does the relative contribution of antibody derived from serum and mucosal sources.

1.11 Restricted gene region usage in anticapsular antibodies

Two experimental approaches can be used to characterise the genetic makeup of the immune response to Hib: direct sequence analysis of monoclonal antibodies, and serological analysis using antibodies directed against the antigen combining site. Genetic studies of antibodies reactive against PRP in adults showed marked uniformity of heavy (V_H) and light (V_L) chain variable region gene usage between individuals. Four V_H regions and 12 V_L regions were commonly expressed. This consistency of genetic composition led to the description of 'canonical' combinations of V_H and V_L segments (Adderson EE et al 1992) (Adderson EE et al 1993) (Lucas AH et al 1999). V_H genes used are of the V_HIIIa and V_HIIIb families, in equal distribution. Of these, the V_HIIIa regions most commonly

combine with κ light chains, while the V_HIIIb family pair with either κ or λ V_L regions (Lucas AH et al 1999).

The use of serological probes directed against the antigen combining site has allowed extension of these studies to the immune response of infants and children to Hib (Lucas AH et al 1999). Two common cross-reactive idiotypes have been identified. HibId-1 is expressed by anti-PRP antibodies utilising the κII-A2/A18 variable region. HibId-2 is a marker for antibodies containing the λVII variable region (Granoff DM et al 1993) (Lucas AH et al 1999). Idiotypic analysis of the immune response to PRP of both adults and children as young as 18 months using HibId-1 demonstrated a consistent pattern of expression of this most dominant idiotype across all ages studied. Thus, canonical gene usage was seen to be present early in the ontogeny of the immune response to polysaccharides (Lucas AH et al 1990) (Lucas AH et al 1999).

1.12 Development of Hib polysaccharide vaccines

As described in section 1.9 above, antibodies with binding specificity for the serotype b capsule were demonstrated to have bactericidal function against Hib, and provided passive protection in animal models. Bacterial polysaccharide immune globulin (BPIG), which contains high levels of anti-PRP antibody, was subsequently demonstrated to protect humans in high-risk groups against Hib infections (Robbins JB et al 1973) (Santosham M et al 1987). On the basis of these observations, the first Hib vaccines to be developed comprised purified capsular polysaccharide, PRP. These vaccines were found to be safe and immunogenic in adults and children over the age of two years. The peak antibody response achieved following immunisation was greater with increasing age (Robbins JB et

al 1973) (Smith DH et al 1973) (Parke JC et al 1977) (Makela PH et al 1977) (Makela O et al 1987). The previously described hyporesponsiveness of immature B cells in children less than two years of age to carbohydrate antigens limited PRP immunogenicity in those at greatest risk (Makela PH et al 1977) (Kayhty H et al 1984).

As mentioned earlier, PRP is a TI antigen. It further belongs to a subset of polysaccharides known as TI-2 antigens, in which T lymphocytes may play a regulatory but not essential role. Additional features of the immune response to TI-2 antigens include the inability to induce immunologic memory, and an antibody repertoire restricted in both isotype and idiotype. As such, each new exposure to a given TI-2 antigen elicits essentially the same short-lived antibody response, dominated by IgG2 and IgM (Rijkers GT et al 1998). In contrast, 'memory' humoral immune responses are characterised by the formation of populations of long-lived T and B lymphocytes. Ongoing clonal selection favours the survival of B lymphocytes producing antibody of higher binding avidity than is seen immediately following primary immunisation (Rajewsky K 1996). These memory B cells have the capacity to rapidly produce high titres of antibodies with superior function on re-exposure to antigen (Ahmed R et al 1996). The isotype distribution of 'memory' or 'anamnestic' responses is composed of relatively more IgG and IgA than IgM (Ahmed R et al 1996).

In safety and immunogenicity studies, serum anticapsular antibodies declined rapidly following immunisation with PRP, particularly in the youngest children (Kayhty H et al 1984). Following repeat vaccination, antibody titres did not differ significantly from those observed in children receiving a first dose of vaccine at the same age, thus confirming the anticipated absence of priming for memory responses (Kayhty H et al 1984). In infants

aged less than 2 years, immune responses were poor, and IgM antibodies to the polysaccharide tended to predominate. A shift in dominance to IgG and IgA after this age (Kayhty H et al 1983b) possibly reflected natural priming by exposure to Hib or cross-reactive antigens in the older group (Peltola H et al 1977). The post-primary immunisation IgG subclasses observed in children aged 24-83 months were a mixture of IgG1 and IgG2, with a predominance of IgG1 (Granoff DM et al 1988). Idiotypic analysis of antibodies induced by immunisation with PRP revealed no apparent difference from 'natural' antibodies in expression of the HibId-1 and HibId-2 idiotypes (Lucas AH et al 1999).

1.13 Efficacy of Hib polysaccharide vaccines

Phase III efficacy trials of Hib polysaccharide vaccines confirmed the clinical relevance of these attributes of the immune response to a TI-2 antigen. Estimates of efficacy over the first 5 years of life in the US were low, in the order of 40% (Parke JC et al 1977). Large-scale Finnish trials involving approximately 98,000 children aged 3 months to 5 years clarified estimates of age specific efficacy. No protection against invasive Hib infection was observed in children under 15 months. In contrast, of 13 Hib disease cases detected over the first year following immunisation in children older than 15 months, none occurred in the cohort who had received Hib vaccine (Makela PH et al 1977). During the second year of follow-up, some waning of vaccine protection was observed, with 2 Hib cases reported in children immunised at18 months of age or older compared with 5 in the control group, although one apparent vaccine failure had a predisposing risk factor for meningitis (Peltola H et al 1977). After four years, vaccine efficacy in these older children was calculated to be 90% (Peltola H et al 1984). Given the distribution of Hib incidence over the first 5 years of life, it was estimated that immunisation at 18 months of age could result in a 60% reduction in all Hib disease in Finland (Peltola H et al 1984).

Based on the results of these clinical trials, an antibody concentration of 1.0 μg/ml or greater was suggested to be the best correlate of immunisation induced protection against Hib meningitis (Kayhty et al 1983a) (Peltola H et al 1984). This figure was considerably higher than the protective level of 0.15 μg/ml associated with naturally acquired antibody. It was suggested that the difference may be explained by the presence of antibodies to bacterial components other than the polysaccharide following Hib exposure, or inferior binding avidity of antibody induced by PRP immunisation (Kayhty et al 1983a) (Peltola H et al 1984).

Nasopharyngeal carriage of Hib was not affected by immunisation in the Finnish trials. Pre-vaccination prevalence of colonisation was 4.6% in the children who took part, rising two years later to 7.4% in the Hib vaccinated group, and 7.7% in the unimmunised (Peltola H et al 1977).

1.14 Development of Hib conjugate vaccines

The limitations of polysaccharide vaccines as described above - absence of protection in the most vulnerable group of infants, and short-lived immunity, necessitated further vaccine development. These problems can be seen to have arisen from the nature of the Hib capsular polysaccharide itself, which is a TI-2 antigen.

As early as 1929, Avery and Goebel observed that physical coupling of monosaccharides to protein antigens produced immunogenic complexes that elicited antibody production specific to the carbohydrate moiety in rabbits (Avery OT et al 1929). Further studies of antibody production induced by carbohydrates conjugated to a series of protein carriers suggested that distinct receptors (Mitchison NA 1967), perhaps present on different cell populations (Rajewsky K et al 1969) were responsible for recognising the two separate classes of antigenic determinants thus represented. Both T and B lymphocytes were subsequently shown to co-operate in the response to hapten-carrier complexes. Interaction between lymphocyte populations was possible as long as the two antigens were chemically coupled, even when separated by a spacing molecule (Fong et al 1978).

This engagement of T cell help was characteristic of the immune response to protein antigens, which is thus termed T cell dependent (TD). Epitopes of proteins ingested and degraded by antigen presenting cells are expressed on the cell surface in association with major histocompatibility complex (MHC) class II molecules. T cells recognising these antigen:MHC complexes become activated and assist B cells expressing the same protein epitopes to produce antibody through secretion of cytokines and binding of co-stimulatory molecules (Figure 1.4). TD responses are age independent, and induce populations of long-

Figure 1.4: Cellular interactions involved in the immune response to conjugates, figure from Ada G 2001

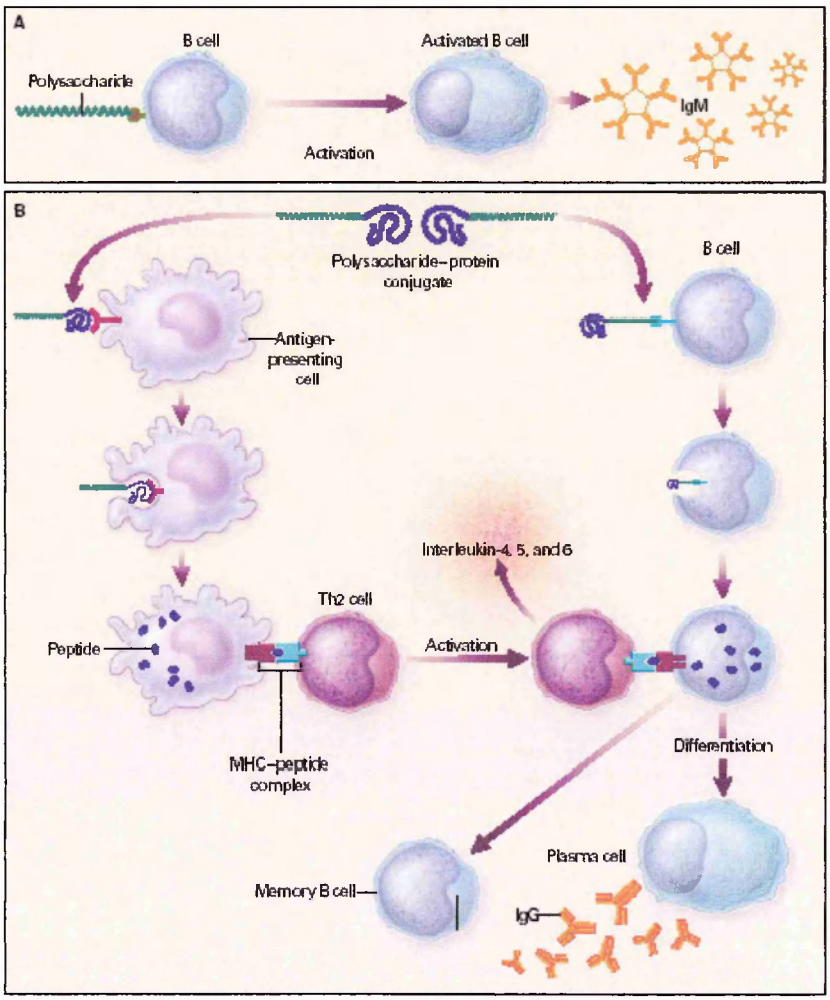


Figure 1 (facing page). Antibody Responses to Polysaccharide Antigens and Polysaccharide–Protein Conjugates.

In Panel A, a polysaccharide antigen binds to an IgM receptor on the surface of a B cell in lymphoid tissues. Once B cells are activated, they produce and then secrete IgM antibody molecules. The individual Fab segments of the IgM molecule have only a moderate affinity, but because there are 10 such segments, an IgM molecule has a high avidity. In contrast, in Panel B, some polysaccharide–protein conjugates will be taken up by dendritic cells, which present peptides from the protein portion of the conjugate to type 2 helper T (Th2) cells. Other conjugate molecules bind to B cells that have IgM receptors specific for the carbohydrate moiety and will undergo endocytosis and be processed by the B cell; the resulting peptides will be expressed with class II MHC molecules on the surface of the B cell. This complex is recognized by the activated Th2 cell, which then secretes interleukin-4, interleukin-5, and interleukin-6. These cause the B cell to differentiate and express IgG molecules with polysaccharide specificity. These cells mature in the lymphoid follicles; only cells that express very-high-affinity IgG molecules become plasma cells and secrete high-affinity IgG that binds strongly to the encapsulated bacteria and mediates opsonic activity and complement-mediated bactericidal activities. A recent study[3] suggests that the formation of memory B cells is a critical component of protective immunity against infection with *Haemophilus influenzae* type b.

lived memory B cells that produce antibodies demonstrating maturation of binding avidity over time (Insel RA 1995) (Rajewsky K 1996) (Nossal GJV 1997) (Goldblatt D 1998a) (Ada G 2001).

This means of converting a TI to a TD antigen was recognised to have potential for application to the production of vaccines against encapsulated organisms (Anderson P et al 1985). Several groups of researchers, working in parallel, developed the first vaccines against Hib based upon the hapten-carrier principle. Smith and Anderson produced a vaccine in which PRP oligosaccharides were directly coupled with CRM_{197}, a mutant diphtheria toxin. The conjugate was more immunogenic than PRP alone in weanling rabbits and primed for memory responses (Anderson P et al 1977b). Schneerson and Robbins established that, when chemically coupled with a range of proteins including diphtheria and tetanus toxoids via a spacer molecule, the immunogenicity of intact PRP polymers in mice, rabbits and monkeys was enhanced (Schneerson R et al 1980, 1984).

Following further studies in animal models and adults, Hib conjugate vaccines incorporating a range of carrier proteins including diphtheria toxoid (PRP-D) (Eskola J et al 1985), CRM_{197} (HbOC) (Anderson P et al 1985, 1987), tetanus toxoid (PRP-T) (Claesson BA et al 1989) and *Neisseria meningitidis* outer membrane proteins (PRP-OMP) (Einhorn MS et al 1986) were shown to be safe and immunogenic in human infants. In addition, antibody responses deemed protective could be elicited in groups of children previously identified to be at increased risk of invasive Hib infection, including those with sickle cell disease or immunoglobulin subclass deficiency (Weinberg GA et al 1990). Conjugate vaccines were also immunogenic in individuals recovered from invasive Hib infection

(Kaplan SL et al 1988), including those who had developed disease despite immunisation with PRP (Weinberg GA et al 1990).

The primary response to conjugate vaccines was dominated by IgG (Granoff DM et al 1988) (Granoff DM et al 1993), with the vast majority of antibodies in infants following primary immunisation belonging to the IgG1 subclass (Kayhty H et al 1988) (Weinberg GA et al 1988) (Granoff DM et al 1988). Boost responses were a mixture of IgG1 and IgG2 (Kayhty H et al 1988) (Weinberg GA et al 1988). Transudation of IgG to mucosal surfaces was observed at high serum antibody levels following vaccination, as measured in saliva (Kauppi M et al 1995). IgA antibodies were also found in saliva. Following primary immunisation these were mainly of the IgA2 subclass, which is usually produced locally in the mucosa. Following boosting in the second year of life, a shift to IgA1 predominance was seen, reflecting increased serum levels of IgA (Kauppi-Korkeila M et al 1998).

Priming for immunologic memory was evidenced by mature for age responses to booster doses of plain PRP at 9-12 months following Hib conjugate immunisation in infancy (Anderson P et al 1985, 1987) (Goldblatt D et al 1998c). These recall responses were later characterised as rapid, detectable within 7-10 days of booster immunisation (Dagan R et al 2001). Further evidence of induction of immunologic memory was inferred in subsequent studies from the demonstration of avidity maturation of antibody over the year following primary vaccination (Goldblatt D 1998d) (Pichichero M et al 1999).

The four Hib conjugate vaccines that have been licensed for use, PRP-D, HbOC, PRP-OMP and PRP-T, varied in their carrier proteins, size and structure of polysaccharide molecules and chemical means of combining the two (Decker MD et al 1998) (Figure 1.5).

Figure 1.5: Structure of different Hib conjugate vaccine formulations, figure from Decker MD et al 1998

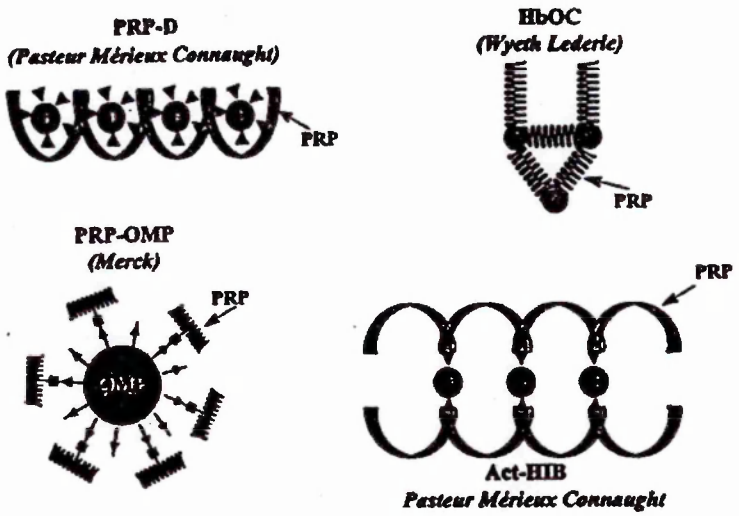


FIG. 1. Differing configurations of the four conjugate Hib vaccines. Shown are the protein carriers (D, C, OMP and T), linking molecules (if any) and short or long chain PRP fragments. See Table 1 for details. Modified from Reference 11.

These differences were reflected in their immunogenicity and ability to prime for memory responses (Lucas AH et al 1999). PRP-D was the least immunogenic preparation in infants and is no longer in routine use in most countries (Decker MD et al 1998) (Weinberg GA et al 1988). Unlike PRP-T and HbOC, PRP-OMP elicited high antibody concentrations following the first dose in infants as young as 2 months of age (Granoff DM et al 1993) (Decker MD et al 1998). It was suggested that its superior immunogenicity was due to OMP acting as both carrier and adjuvant (Perez-Melgosa M et al 2001). While this would appear advantageous, priming with PRP-OMP resulted in only modest memory immune responses on subsequent boosting, far smaller than either of the other two conjugate formulations (Lucas AH et al 1999).

Additional evidence of superior priming for memory by PRP-T and HbOC was demonstrated by a greater focussing of the range of antibody producing cells produced by vaccination. A preponderance of antibodies expressing HibId-1 was seen after primary immunisation with either the tetanus or CRM_{197} conjugate, compared with the production of a wide range of idiotypes in response to PRP-OMP. Following boosting with plain polysaccharide, this difference became more marked (Granoff DM et al 1993) (Chung GH et al 1995) (Lucas AH et al 1999). Minor sequence polymorphisms leading to structural variations in canonical antibodies have been demonstrated to impact on avidity and functional activity (Lucas AH et al 2003). Regardless of the idiotypes produced, evidence of superior avidity maturation leading to improved function was observed following HbOC or PRP-T compared with PRP-OMP (Schlesinger Y et al 1992) (Chung GH et al 1995) (Lucas AH et al 1995). It should further be noted that variation in immunogenicity and functional activity of PRP-T and HbOC between vaccine lots was also demonstrated in these studies (Granoff DM et al 1993) (Chung GH et al 1995).

1.15 Efficacy trials of Hib conjugate vaccines

Phase III trials of PRP-D (Eskola J et al 1987, 1990), HbOC (Black SB et al 1991) (Vadheim CL et al 1994) and PRP-T (Vadheim CL et al 1993) (Booy R et al 1994) showed 83-100% protection against invasive Hib infections in Western children following three doses of conjugate vaccine in infancy. Higher estimates of efficacy were obtained after a booster dose in the second year of life in the studies where initial protection was less than 100%. Consistent with the enhanced immunogenicity profile of conjugate vaccines in infants, vaccine effect was independent of age (Heath PT 1998). A large-scale field trial of PRP-T in the Gambia demonstrated not only 95% protection against classical invasive Hib disease, but also a 21% reduction in all cases of radiologically confirmed pneumonia

(Mulholland K et al 1997). This study demonstrated that Hib was a more common cause of bacterial pneumonia in children than had earlier been thought, suggesting that the impact of Hib vaccines in the developing world could be greater than previously estimated.

Post licensure surveillance of Hib conjugate vaccines has shown near eradication of disease in the short term in almost all Western countries which have implemented them including the US (Shinefield HR et al 1995) (Bisgard KM et al 1998), Canada (Wenger JD 1998), the United Kingdom (UK) (Slack MPE et al 1998) the Netherlands (vanAlphen L et al 1997) and Scandinavia (Jonsdottir KE et al 1992) (Peltola H et al 1999) (Garpenholt O et al 2000). Unfortunately, high cost of the vaccine has limited its global distribution. Consequently, it is estimated that as little as 8% of Hib disease is prevented worldwide by immunisation, a figure which falls to 2% if radiologically confirmed pneumonia believed to be attributable to Hib is included (Peltola H 2000).

At a population level, a marked and unanticipated indirect effect of immunisation was observed due to the ability of Hib conjugate vaccines to delay acquisition of oropharyngeal carriage of the organism in infancy (Murphy TV et al 1993) (Barbour ML et al 1995) (Adegbola RA et al 1998) (Forleo-Neto E et al 1999) (Fernandez J et al 2000). This effect on acquisition was relatively short-lived in the face of ongoing community transmission of the organism. Children vaccinated as babies in early phase II studies of Hib conjugate vaccines were demonstrated to carry at the same prevalence as unvaccinated children by pre-school age. Interestingly, the density of colonisation in the immunised group, assessed by quantitative methods, appeared to be reduced (Barbour ML et al 1993).

With widespread vaccine uptake, however, a sustained reduction in Hib colonisation prevalence was seen in a wide range of countries including Finland (Takala AK et al 1991), the US (Mohle-Betani JC et al 1993) and the UK (Heath PT et al 1997). Subsequent interruption of transmission resulted in a decrease in the incidence of invasive Hib infections in all age groups, even those too young or old to be immunised. This 'herd immune' effect was most marked initially in the UK, where the introduction of routine infant vaccination was augmented by a catch-up campaign involving all children less than four years of age. Hib disease incidence was subsequently observed to fall rapidly in all age groups within twelve months (Rushdy A et al 1999). This was in contrast to the situation in the Netherlands where catch-up immunisation was not employed, and disease rates in the unvaccinated cohorts declined slowly over several years (vanAlphen L et al 1997).

Serological correlates of protection against Hib had been defined for immunity acquired naturally and following polysaccharide vaccination (Kayhty H et al 1983a). As Hib conjugate vaccines do not only produce short-lived antibody responses, but prime for immunologic memory, reliable markers correlating with efficacy were more difficult to define (Plotkin SA 2001). Figures published from the UK in 2000 showed that Hib antibody concentrations waned rapidly following primary immunisation, without apparent loss of vaccine impact. Ongoing clinical protection was attributed to a combination of immunologic memory and herd immunity (Heath PT et al 2000). Persistence of boost responses to PRP was observed at 4-5 years (Scheifele DW et al 1999) and 9-10 years (Makela PH et al 2003) of age in children primed in infancy with conjugate vaccines. Such demonstration of memory immune responses, with maturation of avidity, was considered by some to be sufficient to expect efficacy (Kayhty H 1994) (Granoff DM 2001).

Functional measures of the immune response were thought to be more predictive of disease prevention within an individual than the serum antibody concentration per se, but these were time consuming and more difficult to employ on a large scale (Granoff DM et al 1995).

Definition of protective correlates was also complicated by the strong indirect protective effects associated with widespread immunisation. Interruption of Hib transmission through a reduction in carriage meant that the protection afforded to an individual by vaccination was greater than could be estimated by serum antibody concentrations alone (Kayhty H 1994). It was further difficult to determine the critical antibody concentrations required to maintain herd immunity. One study attempted to define serum IgG anticapsular antibody concentrations associated with reduced Hib carriage (Fernandez J et al 2000). Based on the correlation between post immunisation antibody concentrations with documented colonisation several months later, a protective titre of 5 μg/ml or greater was suggested. Given the low levels of antibody observed in countries like the UK in association with persistent herd immunity (Heath PT et al 2000), it is likely that this threshold was an overestimate.

1.16 A cautionary tale: Hib conjugate vaccines in Alaska

The fallibility of conjugate vaccines has been evidenced in specific high-risk populations. PRP-D vaccine was estimated to be 83% effective in preventing invasive Hib infections after 3 doses in Finnish infants (Eskola J et al 1987). In contrast, the observed efficacy in Alaskan native infants in a similarly conducted study was only 35% (Ward JI et al 1990). Prior to vaccination, this ethnic population experienced extremely high rates of disease with an observed incidence of 332 cases per 100,000 in children under 5 years of age (Singleton R et al 2000). The incidence of early-onset disease was as high as 2960 per 100,000 in the first year of life among infants in the Yukon Kuskokwim Delta (Singleton RJ et al 1994). The more immunogenic conjugate vaccine PRP-OMP proved to be more effective than PRP-D in this population, reducing invasive Hib incidence to just 17 per 100,000 under 5 years of age after its introduction in January 1991 (Singleton R et al 2000).

Following this initial success, however, an alarming resurgence of disease was observed among Alaskan native children in 1996-1997, predominantly in age-appropriately immunised children. Associated factors were a recent switch to primary immunisation using HbOC (Lucher LA et al 1999) and evidence of high prevalence of ongoing nasopharyngeal carriage among children living in rural areas (Singleton R et al 2000). No evidence of clonal shift among Hib strains isolated from carriers or cases, which would be suggestive of changing virulence of the organism, could be found (Lucher LA et al 2002). The conclusion was made that the poorer immunogenicity of HbOC after one or two doses had left infants vulnerable to acquiring Hib infection in the face of high transmission pressure. It was once again recommended that PRP-OMP be used for initial immunisation of all infants (Bulkow LR et al 1999) (Singleton R et al 2000). It remained unclear which

age groups were the primary reservoir for ongoing Hib colonisation in these communities (Dargan JM et al 2000).

This experience highlighted the importance of tailoring vaccine delivery to suit the observed pre-vaccine epidemiology of invasive Hib infections within a given population. It also warned against assuming equal efficacy of immunisation in all settings when predicting possible vaccine impact.

1.17 Conclusion

Hib is an organism that commonly colonises the upper respiratory tract of humans. More rarely, it invades into the bloodstream, producing localised infections including meningitis and epiglottitis, making it a significant cause of morbidity and mortality worldwide. The ability of the serotype b capsule to prevent activation of the alternative complement pathway is postulated to be a major virulence determinant. This assertion is strengthened by the observation that antibodies directed against the capsular polysaccharide PRP correlate with bactericidal activity in serum.

Infants and children under the age of two years exhibit immune hyporesponsiveness to carbohydrates including PRP, which are TI antigens. This places them at greater risk of developing serious infection due to Hib. In consequence, the first Hib vaccines to be developed based on the capsular polysaccharide were ineffective in preventing disease in this group of children at greatest risk. Conjugate vaccine technology was able to overcome this limitation. Chemical coupling of PRP with a range of protein carriers converted it to a TD antigen, to which infants could mount a specific immune response. TD antigens prime for immunologic memory, characterised by maintenance of populations of long lived B

lymphocytes which rapidly produce highly functional antibody on re-exposure to the priming antigen. Antibodies induced in infants by conjugate immunisation appear identical to those resulting from natural exposure to Hib.

In keeping with their enhanced immunogenicity, Hib conjugate vaccines have proven highly efficacious in the prevention of invasive disease both in large-scale vaccine trials and following widespread implementation in whole populations. An additional indirect benefit of immunisation was conferred by the ability of conjugates to delay acquisition of Hib carriage, thus reducing ongoing transmission. A note of caution was introduced by reports of a resurgence of Hib disease in Alaskan native infants. The initiating event was a switch to the use of a less immunogenic conjugate vaccine formulation 5 years after Hib immunisation was introduced. This experience highlighted the fallibility of vaccines and the importance of tailoring the scheduling and type of conjugate vaccine used to underlying epidemiology.

Chapter 2 – Implementation of the Hib vaccine schedule in the UK – epidemiologic consequences

2.0 Introduction

Conjugate vaccines against Hib were introduced into the UK's routine infant immunisation schedule in October 1992. In contrast to many other developed countries, the vaccine was administered as an accelerated primary infant course at 2, 3 and 4 months of age, without a booster dose. Between 1992 and 1993, a 'catch-up' immunisation scheme for children under four years of age supplemented the vaccine's implementation (O'Brien H 1994). Despite an initial dramatic reduction in invasive Hib disease in all age groups, a resurgence of infections was noted from 1999 onwards. The number of reports of serious Hib disease almost doubled each year from that time until 2002, with the majority of cases occurring in fully immunised children (Trotter CL et al 2003a).

2.1 Hib vaccine introduction and scheduling in the UK

The UK has changed the timing of its primary infant immunisation schedule several times over the last forty years. In the early 1960s, an accelerated three dose primary course of diphtheria, tetanus and pertussis was given followed by a booster in the second year of life. In response to low uptake of the fourth dose, the fourth dose was dropped and the schedule lengthened to 3, $4^1/_2$-5 and $8^1/_2$-11 months of age to maximise immunogenicity. This decision was made despite concerns that attendance for immunisation was poorest after 6 months of age (Ramsay ME et al 1991) and therefore in May 1990 to further improve immunisation coverage the switch was made back to the accelerated schedule at 2, 3 and 4 months without a subsequent booster (Ramsay ME et al 1991). Comparable immunogenicity and safety of both regimens was confirmed in controlled studies (Ramsay

ME et al 1992, 1993). In addition, antibody titres deemed protective against diphtheria, tetanus and pertussis were demonstrated to persist for 4 years after an accelerated course completed by the age of 6 months (Ramsay ME et al 1991).

Conjugate Hib vaccines were introduced into this infant schedule in October 1992, to be given concomitantly with the triple vaccine. In a relatively unique decision for a developed country, no booster dose was included (http://www.hpa.org.uk/infections/topics_az/vaccination/vac_sced.htm). In North America, Hib was scheduled for use at 2, 4 and 6 months of age, with a fourth dose at 12-18 months (http://www.cdc.gov/nip/recs/child-schedule.PDF). In Scandinavian countries, the primary course was most commonly 2 doses, at 3 and 5 or 4 and 6 months, with a booster late in the first or early in the second year of life (Peltola H et al 1999). While no formal policy statement regarding the absence of a fourth dose accompanied vaccine introduction in the UK, subsequent authors attributed the lack of need for a booster to the conjugate vaccine's ability to prime for immunologic memory (Booy R et al 1997).

Over the first 12 months of Hib vaccine implementation, 'catch-up' immunisation was recommended for all children under 48 months of age. Those less than 13 months old were given 3 doses of vaccine and older cohorts received a single dose (O'Brien H 1994). Coverage data from a single child health record's system in the North East Thames region provided the best available estimate of national catch-up immunisation uptake (O'Brien H 1994). Quarterly rates of coverage achieved in relation to vaccination targets are shown in Figure 2.0. By the end of 1993, uptake by birth cohort over the first year of the campaign was as follows: 1989 (3-4 year olds) – 34%, 1990 (2-3 year olds) – 77%, 1991 (1-2 year olds) – 87% and 1992 (6 month-1 year olds) – 89% (O'Brien H 1994).

Figure 2.0: Cumulative coverage of Hib catch-up immunisation in North East Thames by birth cohort and quarter (from O'Brien H, 1994).

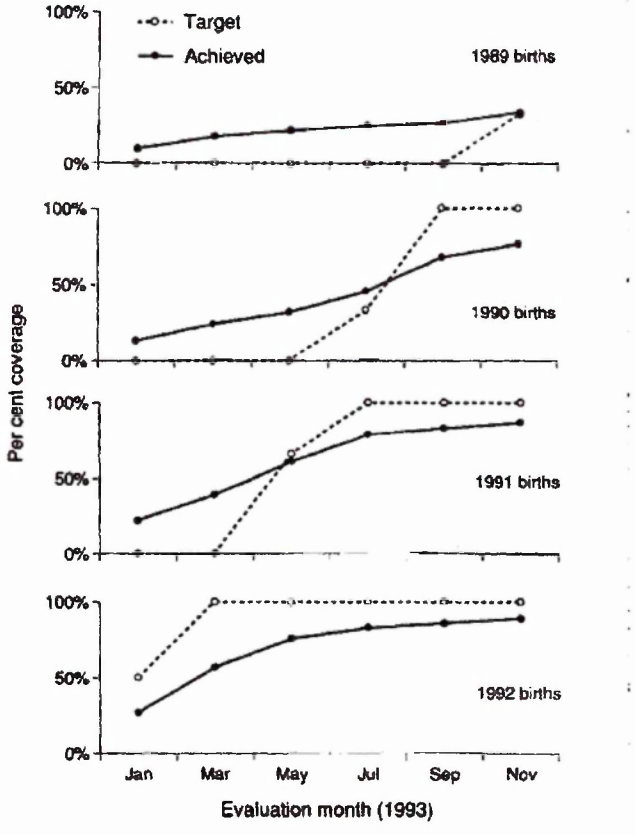
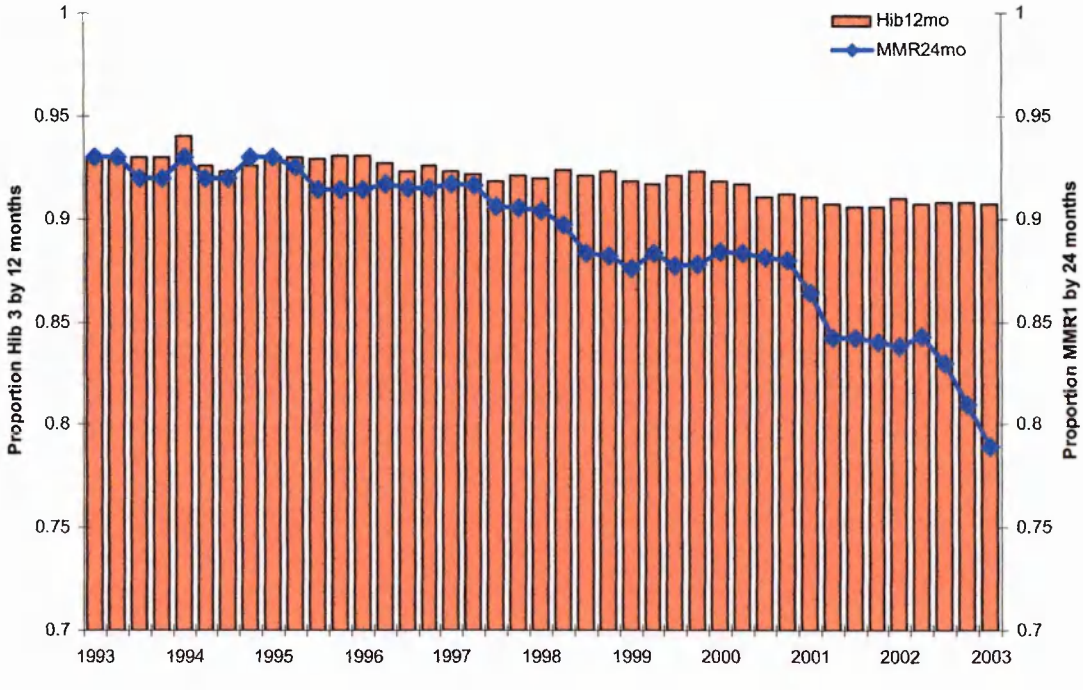


Figure 2.1: Uptake of 3 doses of Hib vaccine by 12 months of age, compared with a single dose of MMR vaccine by 24 months of age (source: COVER data, http://www.hpa.org.uk/cdr/archive04/immunisation04.htm)



2.2 Hib vaccine coverage in the UK 1993-2003

High rates of Hib vaccine coverage for the infant primary course have consistently been achieved over the past 10 years as recorded in the Coverage of Vaccination Evaluated Rapidly (COVER) statistics collated quarterly throughout the UK by the Health Protection Agency (HPA) (http://www.hpa.org.uk/cdr/archive04/immunisation04.htm). Figure 2.1 shows uptake of 3 doses of Hib by the first birthday in the UK, by quarterly birth cohorts. Adverse publicity surrounding the measles, mumps and rubella (MMR) vaccine (Taylor B et al 1999) has resulted in declining coverage for a single dose of this vaccine by 2 years, now below 80% (Figure 2.1). No such impact has been observed on the other vaccines in the primary infant immunisation schedule.

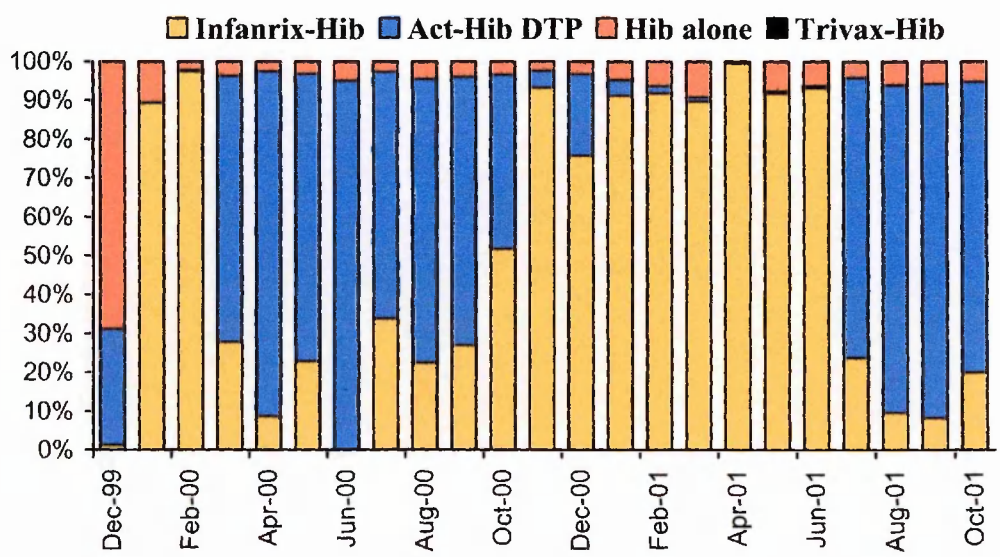
2.3 Hib conjugate vaccine formulations used in the UK

A number of conjugate vaccine formulations have been licensed for use in the UK over the past 10 years (Dr M Ramsay, Immunisation Department, HPA, personal communication) (Table 2.0). Different manufacturers' vaccines were used for catch-up and routine immunisation in 1992. Hib was administered in a separate syringe as a single antigen until 1996, after which time it became available for use as one injection with diphtheria, tetanus and whole cell pertussis vaccines (DTwP-Hib). A global shortage of DTwP-Hib necessitated the use of a corresponding preparation containing an acellular pertussis vaccine (DTaP-Hib), which was distributed in the UK after December 1999 (Figure 2.2). Glaxo SmithKline withdrew Trivax-Hib from sale in the UK in 2000.

Table 2.0: Hib conjugate vaccines licensed for use in the UK, 1992-2003.

Year Licensed	Type of Conjugate	Vaccines in combination	Trade Name	Manufacturer
1992 (infant)	PRP-T	Nil	Act-Hib	Aventis Pasteur
1992 (catch-up)	HbOC	Nil	Hibtiter	Wyeth
1996	HbOC	mixed with DTwP	Hibtiter	Wyeth
1996	PRP-T	DTwP	Act-Hib DTwP	Aventis Pasteur
1997	PRP-T	DTwP	Trivax-Hib	Glaxo SmithKline
1999	PRP-T	DTaP	Infanrix-Hib	Glaxo SmithKline

Figure 2.2: Hib vaccine formulations distributed in the UK by month, December 1999 to October 2001. Source: Dr Mary Ramsay, Immunisation Department, HPA



2.4 Impact of Hib immunisation on invasive disease in the UK

Literature Review

Prior to the introduction of Hib vaccination, the Public Health Laboratory Service (PHLS) established a Regional Survey of invasive Hi disease (Anderson EC et al 1995). The purpose for this network of microbiology laboratories was to provide an accurate assessment of the baseline Hib disease burden in the UK, from which to monitor the impact of introducing routine immunisation. The Survey was initiated in Oxford in 1985, extended to Wales in 1988 and was expanded to include four additional healthcare regions (East Anglia, Northern, North-Western and South-Western) in 1990. The population denominator thus represented was approximately 35% of all England and Wales (Anderson EC et al 1995).

The case definition of invasive Hi infection required isolation of the organism from a normally sterile site, or detection of Hib antigen in conjunction with Gram-negative organisms on Gram stain. Strains were typed in local laboratories, and sent to one of two centralised reference laboratories (Oxford in England, Bangor in Wales) for confirmatory identification by immunotyping and polymerase chain reaction (PCR). Reports were compared with two additional sources of data in order to maximise ascertainment. These were notifications of meningitis obtained by the Office for National Statistics (ONS), and all invasive Hi disease reported directly to the PHLS Communicable Disease Surveillance Centre (CDSC) (Anderson EC et al 1995).

Of almost 1,000 reports of invasive Hi infections made over a 2-year period, 82% were due to Hib. The age specific incidence of Hib disease calculated in this survey is shown in Figure 1.2 of Chapter 1. As in other developed countries, children between 6 months and 2

years of age were at highest risk, with a decline in incidence thereafter. In all, 88% of notified cases occurred by the age of 5 years. The distribution of clinical presentations by age group is shown in Table 2.1. Hib most commonly caused meningitis in children and pneumonia in adults. Epiglottitis was relatively rare in children less than 2 years of age. The case fatality rate for all Hib infections was 3.9% (95% CI 2.6-5.5), with most deaths in neonates and the elderly (Anderson EC et al 1995). These patterns were similar to those previously reported from the Oxford region alone (Booy R et al 1993).

Table 2.1: Clinical presentation with invasive Hib infection, by age group, PHLS Regional Survey (from Anderson EC et al 1995)

Clinical Diagnosis	Total Cases	% of all cases	No cases < 2 yrs	% of cases < 2 yrs	No cases 2-15 yrs	% of cases 2-15 yrs	No cases >15 yrs	% of cases > 15 yrs
Meningitis	437	56	314	65	111	52	8	12
Epiglottitis	111	14	34	7	66	31	10	15
Pneumonia	41	5.5	13	3	7	3	21	31
Cellulitis	54	7	50	10	3	1.5	1	1.5
Septic arthritis	37	5	24	5	11	5	2	3
Osteomyelitis	6	<1	6	1	0	0	0	0
Bacteraemia	63	8	27	6	15	7	18	27
Other	20	2.5	13	3	0	0	7	10
Unknown	3	<1	2	<1	1	<1	0	0
Total	772	100	483	100	214	100	67	100

2.4.1 Overall trends in disease presentation after vaccine introduction

Methods

Following Hib vaccine introduction, surveillance for invasive Hi infections was extended beyond the regional survey to include all of the UK and Republic of Ireland. As before, local laboratories were asked to refer strains to central laboratories in England and Wales to confirm identification by serotyping and PCR. From 1995 onwards, all isolates were sent to a single *Haemophilus* Reference Unit (HRU), based at the John Radcliffe Hospital in Oxford (Trotter CL et al 2003a). For isolates not referred to the HRU, the source laboratory typing results were used. In order to meet the case definition for the purposes of this analysis, Hib had to be cultured either from a normally sterile site or from an upper airway swab in a patient presenting with clinical epiglottitis.

Additional clinical reports were made through the orange card reporting system of the British Paediatric Surveillance Unit (BPSU) between 1992 and 2000. Every paediatrician in the UK received a monthly reporting card asking whether one or more of a number of notifiable diagnoses had been seen in the preceding month. From 1992 to 1995, clinicians were asked to report invasive Hi infections in vaccinated children, and in all children regardless of vaccination status from 1995 onwards. Cases identified in this way were followed up by the HRU in order to obtain an isolate, if possible.

The BPSU study was conducted in collaboration with the Oxford Vaccine Group (OVG) of the University of Oxford Department of Paediatrics, who had a particular interest in clinical and immunological risk factors associated with Hib vaccine failure (Booy R et al 1997). Ethical approval for the study was obtained from the Central Oxford Research

Ethics Committee in 1991, and again from the South-East Multi-Centre Research Ethics Committee in 2001 (McVernon J et al 2003b).

Data represented in the figures below were derived from a single reconciled *Dataease* dataset maintained by the HPA's HRU and CDSC. Reports of cases presenting to the end of June 2003 were included, as downloaded in September 2003. Due to less consistent ascertainment from areas of the UK outside England and Wales, figures were reported from these two countries only.

Results

The distribution of all invasive Hi reports, by typing status and serotype, is shown in Figure 2.3. The proportion of isolates typed over the period improved from 70% in 1990 to 80% in 2002. This increase reflected greater referral of strains to the HRU with time. Hib's relative contribution to the total burden of Hi infections fell sharply after 1992, remaining low to 1998, but rose again subsequently.

A dramatic reduction in all invasive Hib infections was observed following introduction of widespread vaccination late in 1992. This effect was not restricted to children of an age eligible for immunisation (Figure 2.4). Excellent disease control was maintained until 1999, when the number of cases began to rise, most markedly in children aged 1-4 years, and adults 15 years or older. Despite a steep increase in paediatric reports between 1999 and 2002, numbers remained well below those observed prior to vaccine use.

Figure 2.3: Serotype distribution of all invasive Hi reports, 1990-2002

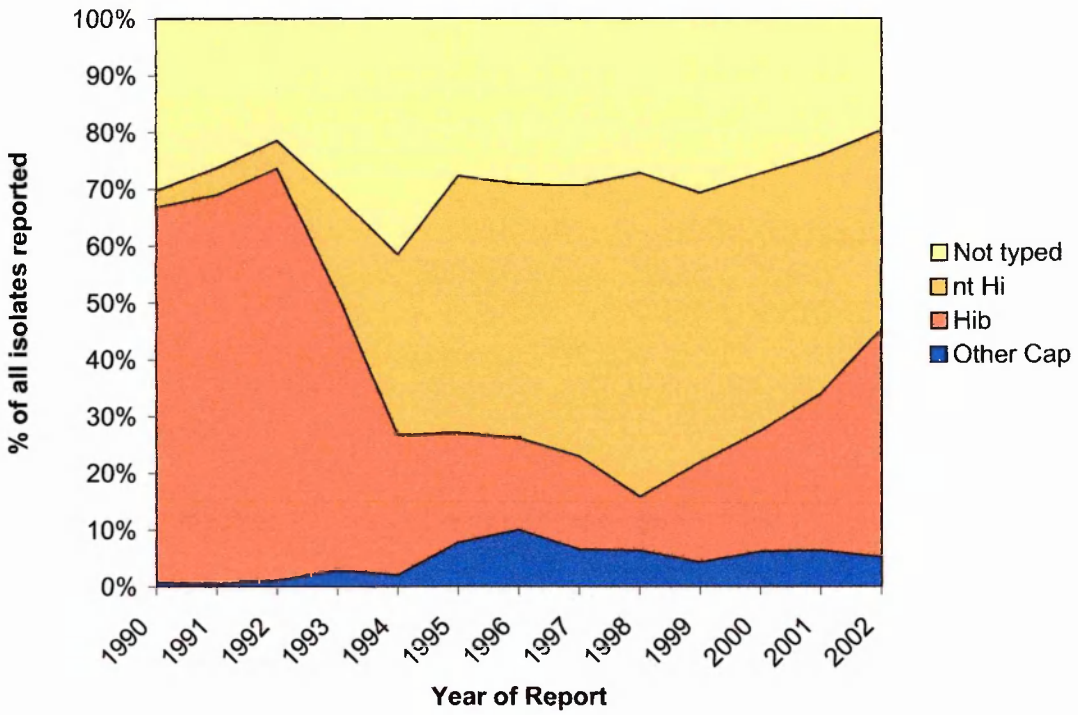
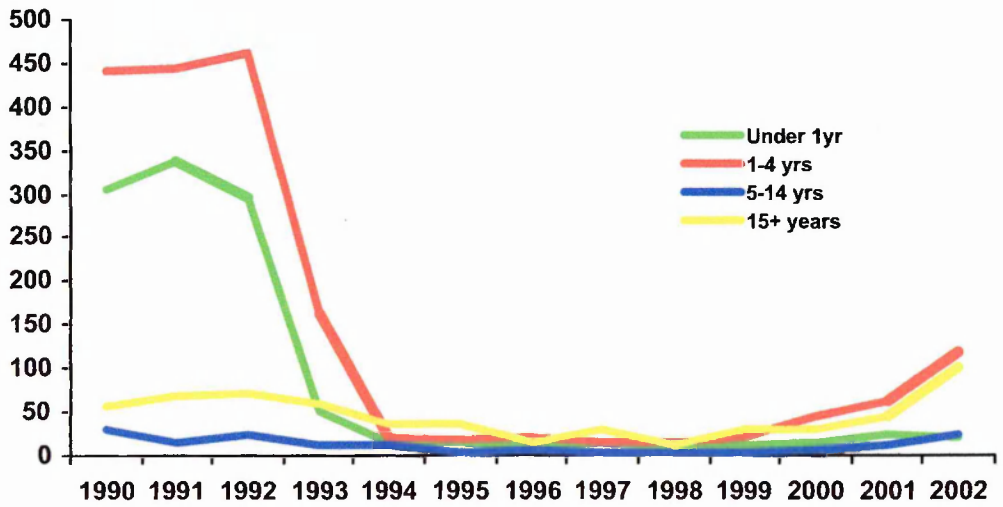
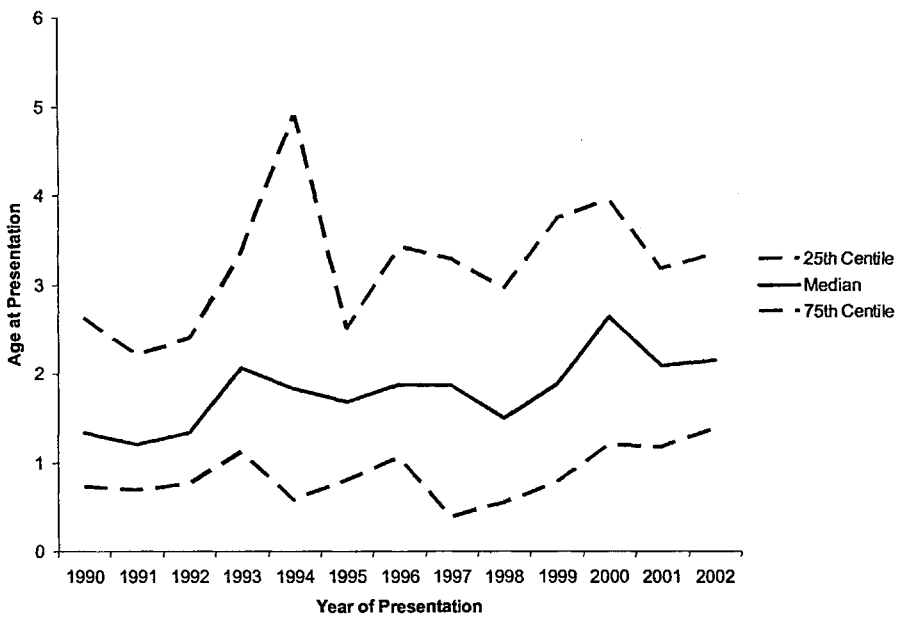


Figure 2.4: Invasive Hib infections by age group, England and Wales 1990-2002. Combined CDSC/HRU dataset.



The most marked impact of vaccine on disease reduction was seen in children less than two years of age. Figure 2.5 shows the median paediatric (<15 years) age at presentation with Hib, by year, with interquartile ranges. An increase in the most common age at disease from the second to the third year of life reflected high vaccine efficacy in the youngest infants. There was no corresponding shift of the burden of Hib to older age groups (5 years and older) who had not previously experienced high rates of invasive infection.

Figure 2.5: Median age at presentation with invasive Hib infection, 1990-2002. Broken lines represent interquartile ranges.



Clinical presentations of invasive Hib infection by age group over the period 1993 to 2002 are given in Table 2.2. Data are presented according to the same age divisions used in Table 2.1, describing the condition pre-vaccination. Apart from an increase in the proportion of cases presenting with bacteraemia in the paediatric age groups, no change in the distribution of age specific presentations was observed. It is likely that the increased diagnosis of bacteraemia may reflect an increasing clinical trend to take blood cultures

from febrile children in the UK in recent years, rather than representing any real epidemiological change.

Table 2.2: Clinical presentation with invasive Hib infection by age group, England & Wales, 1993-2002

Clinical Diagnosis	Total Cases	% of all cases	No cases <2 yrs	% of cases <2 yrs	No cases 2-15 yrs	% of cases 2-15 yrs	No cases >15 yrs	% of cases >15 yrs
Meningitis	348	33	203	59	121	33	24	7
Epiglottitis	178	17	19	5	93	26	66	18
Pneumonia	119	11	10	3	13	4	96	27
Cellulitis	32	3	19	5	7	2	6	2
Septic arthritis	32	3	17	5	8	2	7	2
Osteomyelitis	1	<1	1	<1	0	0	0	0
Bacteraemia	182	17	40	12	59	16	83	23
Other	174	16	37	11	61	17	76	21
Unknown	1	<1	1	<1	0	0	0	0
Total	1067	100	347	100	362	100	358	100

The overall Hib case fatality rate rose from 3.9% to 7.5% (95% CI 5.9, 9.5) with widespread vaccine use. It had previously been observed that mortality from Hib is highest in infants under 6 months of age and the elderly (Anderson EC et al 1995). In this dataset, the death rate in children aged 6 months to 15 years was 4.4% (2.9, 6.5), rising to

13.3% (9.5, 18.0) in adults 15 years and older. With a fall in paediatric cases, the relative contribution of adult data to mortality statistics was greater, thereby increasing the overall proportion of cases in which death was the outcome. Of particular importance, there was no statistically significant difference in death rates between vaccinated [3.6% (2.2, 5.6)] and unvaccinated [4.6% (1.5, 10.4)] children with invasive Hib infection.

2.4.2 Incidence of invasive disease in children under 5 years of age, vaccination status and duration of protection by vaccination

Methods

Numbers of disease reports by age for children under 5 years in England and Wales were converted to incidence rates using annual population denominators reported by the ONS (http://www.statistics.gov.uk). Because of the change in case ascertainment through the BPSU study in 1995, when unvaccinated as well as vaccinated cases were included, only figures from 1996 onwards were included to ensure comparability.

Vaccination status of Hib cases was confirmed by contacting the child's usual general practitioner, or from computerised records held in local child health departments. Immunisation dates and batch numbers were collected and stored on the CDSC/HRU database. From this information, the proportion of children presenting with Hib infection could be calculated who had received three doses of vaccine in the first year of life, prior to onset of disease.

Figure 2.6: Annual incidence of invasive Hib infections in children less than 5 years of age, England and Wales reports to June 2003, combined CDSC/HRU dataset. (Percentage in brackets refers to proportion of cases occurring after three doses of vaccine in infancy)

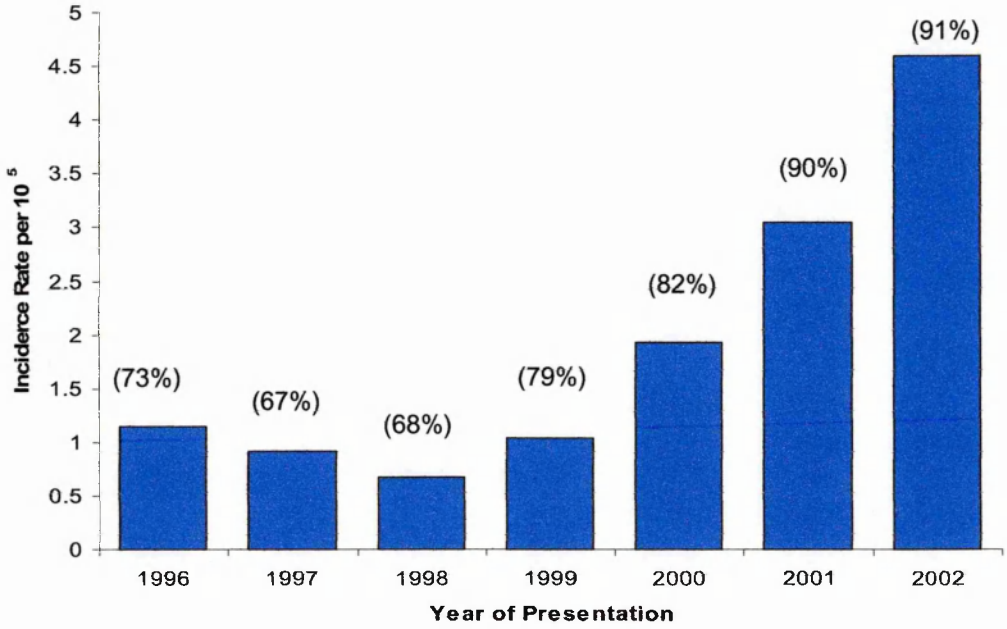
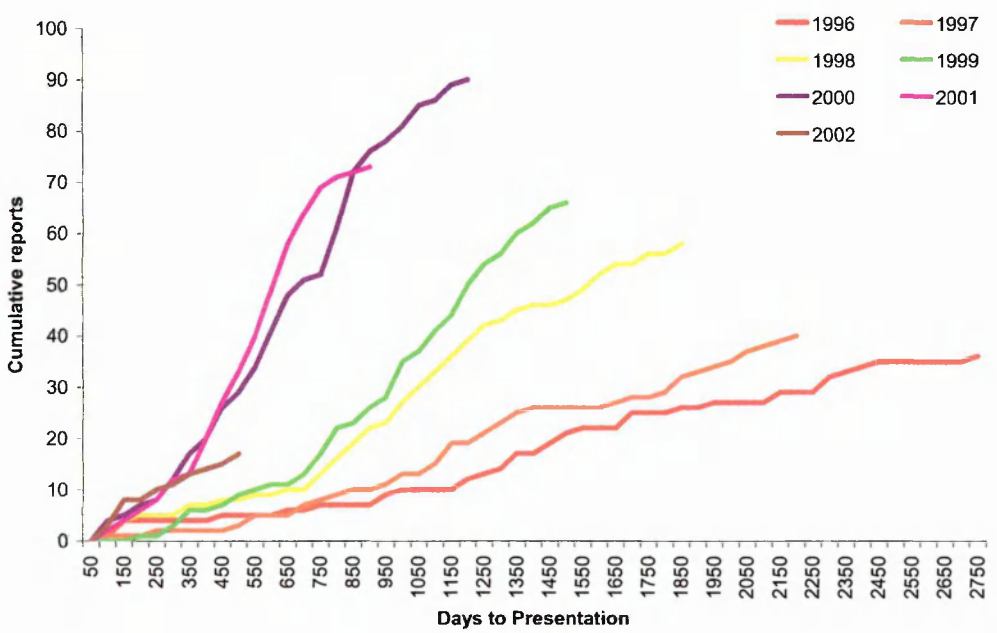


Figure 2.7: Cumulative number of vaccinated children presenting with invasive Hib infection in each birth cohort year by age in days: England and Wales reports to June 2003, combined CDSC/HRU dataset.



Age at onset of disease was calculated for vaccinated children with confirmed invasive Hib infection. Results were grouped by year of birth cohort, in order to compare the duration of protection conferred by the vaccine over time.

Results

The incidence of invasive Hib infection in children less than 5 years of age in England and Wales is shown in Figure 2.6. Following a nadir of 0.68 per 100,000 in 1998, disease incidence rose steadily to 4.6 per 100,000 in 2002. As can be seen from the proportion of children presenting each year who had been fully immunised, the majority of the increase in disease occurred in the vaccinated proportion of the population, rising from 68% in 1998 to 91% in 2002.

Figure 2.7 plots the cumulative number of Hib reports arising from children born in sequential years. Changes in the duration of protection conferred by immunisation can be seen in birth cohorts as early as 1998. An increase in the number of cases was observed in children born each year from 1998 to 2000. In addition, the onset of disease was earlier in each successive group, with a marked reduction in the 2000 birth cohort. While very few children born in 2002 had presented with invasive Hib infection by the end of June 2003, the data shown suggest some improvement in vaccine protection compared with those born in 2000/2001.

2.4.3 Incidence of invasive disease in adults 15 years of age and over

Methods

Case ascertainment for invasive Hi in adults was as detailed above, with the exception of clinical reporting through the BPSU study. Presumably due to a lower clinical profile of

Hib disease in unvaccinated age groups, referral of adult isolates to the HRU over time was initially poor at just under half in 1991, improving to 73% in 2002. As greater consistency of reporting was observed from the five enhanced surveillance regions (East Anglia, Northern, North West, South West and Oxford) involved in the Regional Survey, these data are represented separately. In a further attempt to correct for changes in typing of adult Hi isolates, the following formula was used when examining the entire dataset for England:

$$\textit{Adjusted Hib Reports} = \textit{Known Hib Reports} + \left(\textit{All Typed Strains} \times \frac{\textit{Known Hib Strains}}{\textit{Untyped Strains}} \right)$$

Results

A total of 3,408 adult invasive Hi infections were reported over the period 1991-2002, of which 561 (16%) were due to Hib, 1353 (40%) nt Hi and 1291 (38%) untyped (Table 2.3). Nine cases of Hib infection were included where the isolate was from a non-sterile site with a clinical diagnosis of epiglottitis. The three most common clinical presentations of invasive Hib infection in adults were pneumonia (n=127, 28%), bacteraemia (n=99, 22%) and epiglottitis (n=90, 20%), together constituting approximately two thirds of all reports. The outcome of Hib disease was known for 77% of cases reported between 1991 and 2002; the case fatality rate in adults during each year ranged from 0 to 27%, and was 8.1% in 2002.

A decrease in adult infections due to serotype b was observed following introduction of Hib vaccine in October 1992, falling to 1998 and then rising to 2002 (Table 2.3 and Figure 2.8). This trend was also seen in cases from the five regions with enhanced surveillance

Figure 2.8: Number of adult (over 15 years) Hib reports and median Hib antibody titres with 95% CIs (30-39 year olds) by year, England.

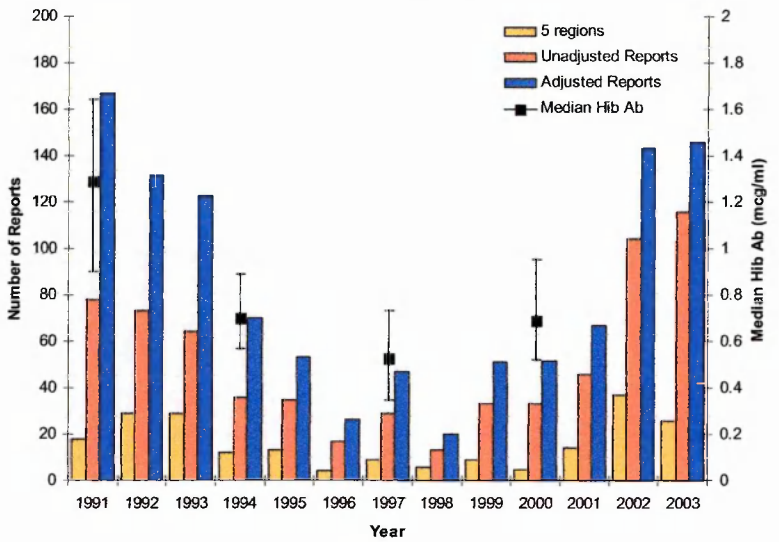


Figure 2.9: Frequency distribution of age at presentation with invasive disease:
a) Non-typeable *H influenzae* **b) *H influenzae* type b**

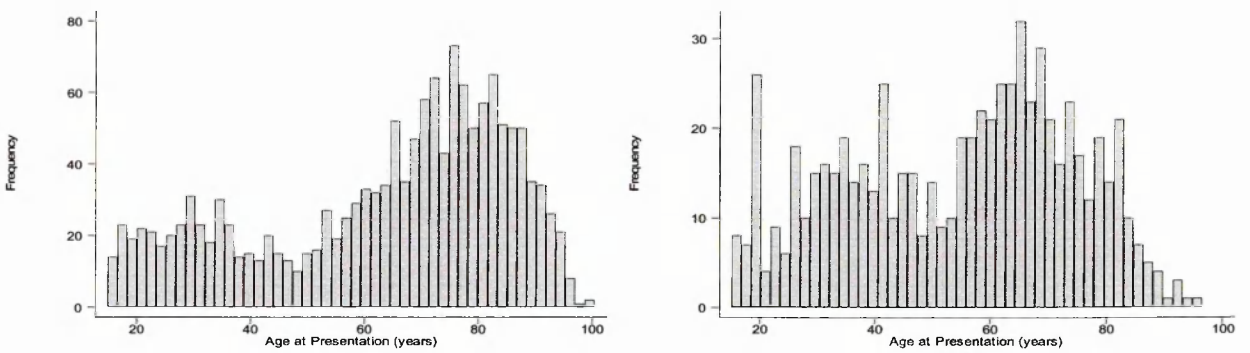
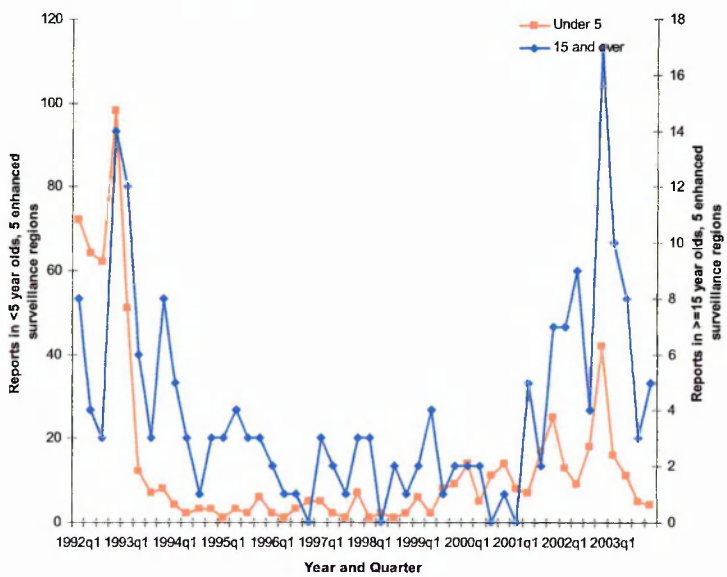


Figure 2.10: Comparison between Adult (>15 years) and Child (<5 years) quarterly Hib reports, 1992-2000, 5 enhanced surveillance regions



throughout the period, and in the national dataset after adjusting for untyped isolates, using the method described above (Figure 2.8).

Table 2.3: Numbers (and percentages) of laboratory reported invasive isolates in adults (over 15 years) by typing status and serotype, combined HRU/CDSC dataset, 1991-2002.

Year of Report	Hib	nt Hi	Other Caps	Untyped	Total
1991	78 (32%)	32 (13%)	4 (2%)	130 (53%)	244
1992	73 (33%)	39 (18%)	10 (4.5%)	98 (44.5%)	220
1993	64 (23%)	71 (25%)	12 (4%)	135 (48%)	282
1994	36 (17%)	71 (32%)	6 (3%)	106 (48%)	219
1995	35 (13%)	120 (46%)	18 (7%)	90 (34%)	263
1996	17 (7%)	105 (45%)	27 (12%)	83 (36%)	232
1997	29 (10%)	121 (44%)	22 (8%)	105 (38%)	277
1998	13 (5%)	150 (54%)	17 (6%)	97 (35%)	277
1999	33 (11%)	142 (49%)	14 (5%)	102 (35%)	291
2000	33 (10%)	155 (47%)	21 (7%)	119 (36%)	328
2001	46 (13%)	171 (49%)	24 (7%)	110 (31%)	351
2002	104 (25%)	176 (41%)	28 (7%)	116 (27%)	424
Total	561	1353	203	1291	3408

Amongst adult cases with a serotype, the age distribution differed between Hib and nt Hi infections (Figure 2.9). While nt Hi was predominantly a cause of serious infections in the elderly, a bimodal distribution in Hib cases could be seen, peaking at 30 and 65 years, ages at which household exposure to children is common (www.statistics.gov.uk/StatBase). In keeping with these observations, the trend in adult infections followed closely the changing incidence of childhood disease (Figure 2.10).

2.4.4 Comparison of laboratory derived invasive disease reports with hospital admissions data for epiglottitis

Methods

Cases of epiglottitis due to Hib in England were identified from the same CDSC/HRU dataset of invasive *Haemophilus* infections described above. In order to meet the case definition for the purposes of this analysis, Hib had to be cultured either from a normally sterile site or from an upper airway swab in a patient presenting with clinical epiglottitis.

In order to gain an idea of completeness of reporting of Hib epiglottitis, figures derived from the microbiology reports were compared with a national dataset describing hospital admissions. English hospital providers submit data annually to the Department of Health regarding inpatient episodes, to comprise the Hospital Episode Statistics (HES) database. Between 1989 and 1994, discharge diagnoses were classified according to International Statistical Classification of Diseases Version 9 (ICD-9) criteria. From 1995 onwards, the corresponding ICD-10 codes have been used. Inpatient admissions for epiglottitis between March 1989 and March 2002 were identified by searching for the following codes from ICD-9: acute epiglottitis 464.3, acute laryngitis and tracheitis 464 and ICD-10: J05.1 Acute epiglottitis, J05 Acute obstructive laryngitis (croup) and epiglottitis.

Two other organisms implicated in the aetiology of epiglottitis in adults are Group A Streptococcus and *Streptococcus pneumoniae* (Trollfors B et al 1998). Laboratory reports of blood isolates of these pathogens in adults 15 years of age and over are routinely compiled by CDSC. Additional surveillance for pneumococcal infections was conducted by the Respiratory and Systemic Infection Laboratory (RSIL) of the Specialist and Reference Microbiology Laboratory from 1996 onwards. These data were compared with

reports of all bacteraemias attributable to *Haemophilus influenzae,* for the years 1990 to 2002.

Results

524 laboratory reports of Hib epiglottitis were received between 1990 and 2002. The proportion of all invasive Hib disease presenting as epiglottitis remained constant at between 10 and 20% of cases, in both children and adults (data not shown). Similarly, there was no obvious increase in case fatality over time, with 13 deaths in children and 7 in adults over the period. These represented rates of 6% and 11%, respectively, for cases in which the survival outcome was known (75% of paediatric and 78% of adult cases). 4374 hospital admissions for infections with a focus in the upper airway were recorded from the beginning of 1990 to the end of March 2002.

Figures 2.11a and 2.11b show the numbers of epiglottitis cases per year in England for children less than 15 years and adults 15 years and over, respectively. Data on the number of laboratory reports of Hib epiglottitis are contrasted with the HES figures. Due to the necessarily broad range of case definitions included in the searches of the hospital datasets, a range of other presentations referable to the upper airway are included in these figures which may not fit a classic case definition of epiglottitis. An initial decrease in the number of paediatric cases is clear from both sources. A reduction in the number of adult reported Hib cases occurred in the mid to late 1990s, rising again in 2002. Less certain is the overall impact of vaccination on all hospital admissions for upper airway infections in adults, which appear to have increased in number in recent years. This is in keeping with the more diverse aetiology of epiglottitis in this age group.

Figure 2.11a: Laboratory reported cases and hospital admissions for epiglottitis in English children <15 years of age, 1990-2002

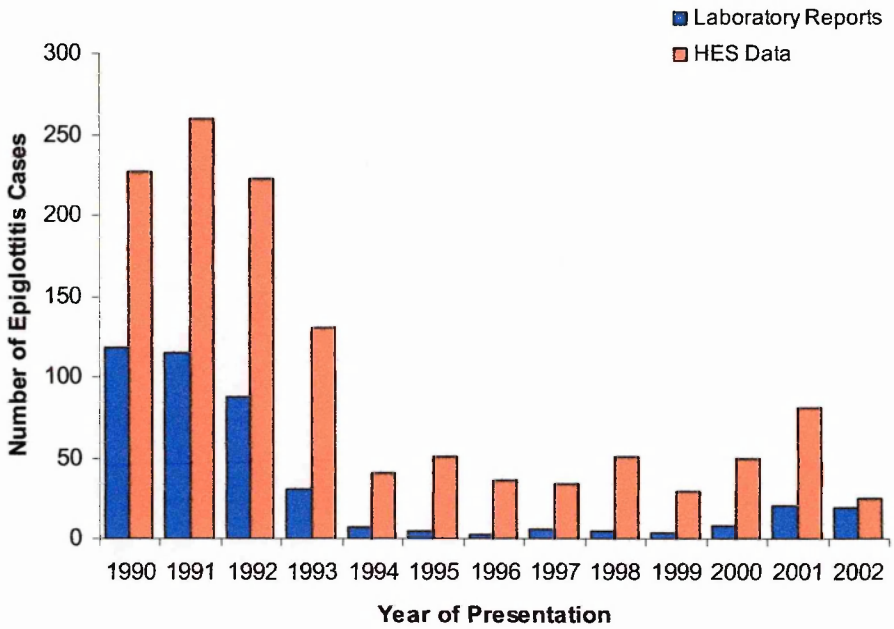


Figure 2.11b: Laboratory reported cases and hospital admissions for epiglottitis in English adults ≥15 years of age, 1990-2002

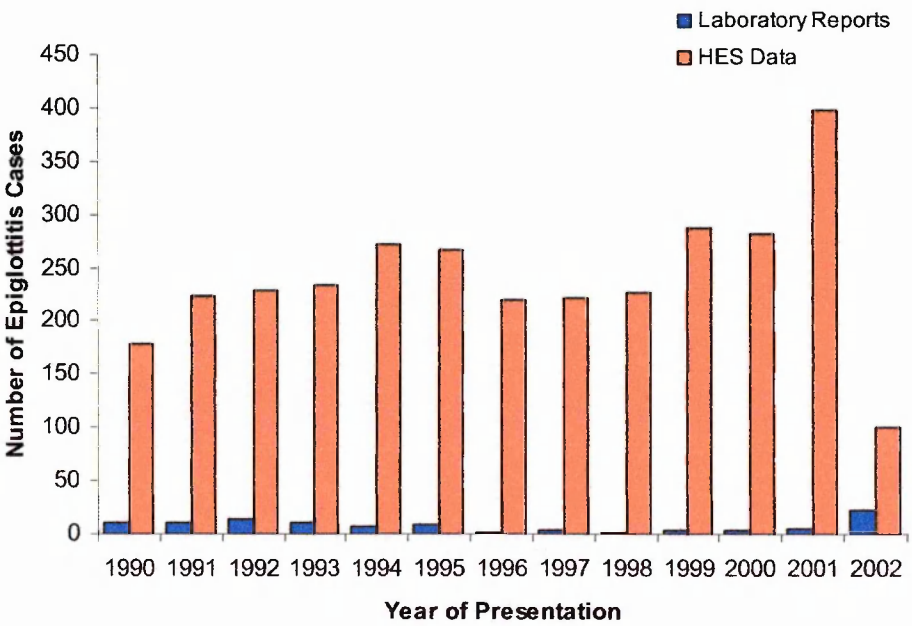


Table 2.4 documents the number of blood isolates of Group A Streptococcus, *Streptococcus pneumoniae* and Hi reported to CDSC and RSIL between 1990 and 2002 in adults 15 years of age and over. An increase in all reports is seen over time. While some of the rise in pneumococcal infections may be attributed to increased ascertainment through RSIL, active follow-up of cases through this laboratory focussed primarily on disease in children under five years of age, and thus would be expected to have a lesser impact on the number of adult isolates identified.

Table 2.4: Blood isolates of Group A Streptococcus, *Streptococcus pneumoniae* and *Haemophilus influenzae* in adults 15 years and over (England and Wales)

Year	H influenzae	Group A Strep	Str Pneumo	Total
1990	163	406	2645	3214
1991	205	408	2948	3561
1992	174	413	2665	3252
1993	222	455	2951	3628
1994	183	416	2849	3448
1995	193	389	3322	3904
1996	177	554	3907	4638
1997	218	558	4302	5078
1998	221	602	3868	4691
1999	221	624	3816	4661
2000	222	763	3800	4785
2001	258	778	3201	4237
2002	321	829	3530	4680

Conclusions – invasive disease

The introduction of routine immunisation against Hib in the UK in October 1992 was initially responsible for a decrease in invasive Hib disease incidence in all age groups (Slack MPE et al 1998). The fall in adult cases mirrored that observed in immunised children and was indicative of a 'herd immunity' effect (Sarangi J et al 2000). While the relative contribution of nt Hi to the overall disease burden of Hi increased in consequence, no evidence of replacement with other capsular serotypes was seen (Heath PT et al 2001).

The greatest impact on invasive Hib infections was noted in the very young, who were at highest risk of disease. An 87% reduction in Hib meningitis in children less than 5 years of age within a year of vaccine introduction has previously been reported (Urwin G et al 1994). Consequently, the median age at Hib disease in childhood increased in the years following vaccine introduction, then plateaued. Of significance, there was no subsequent shift of the burden of infection into older paediatric age groups who had not previously experienced high rates of disease. In addition, no evidence of any change in disease severity was observed in immunised or unimmunised children, as indicated either by the range of clinical presentations or case fatality attributable to Hib.

A worrying increase in serious Hib infections was seen between 1999 and 2002, predominantly among vaccinated children. The incidence of disease in children less than 5 years old rose almost 7 fold to 4.6 per 100,000 over this period, but remained well below the pre-vaccination incidence of 30 per 100,000. Similar trends in hospital statistics describing episodes of admission for epiglottitis in childhood confirmed the validity of the microbiological data, and placed in context earlier case reports of a resurgence in epiglottitis (McEwan J et al 2003). The increase in paediatric cases was associated with

evidence of a reduction in the duration of protection afforded by Hib vaccine from 1998 onwards, with decreasing age at clinical presentation with infection most marked in children born in 2000 and 2001.

After correcting for variability of reporting over time, a corresponding increase in adult Hib disease cases has been noted. Adults of the ages most likely to mix with children, both as parents and grandparents, seem most liable to Hib infections. This observation is not surprising, given the high documented rates of parental carriage in families with a child known to be colonised or recovering from invasive disease due to Hib (Boisvert PL 1948, Michaels RH et al 1977, Barbour ML et al 1995). In 1975, Turk predicted that widespread Hib immunisation may delay Hib carriage, or force circulation into older age groups, so that 'a young mother might pass on to her newborn baby the organism itself rather than [the] passive immunity' (Turk DC 1975). While we believe that transmission is most often from child to parent, the reverse may also occur (Glode MP et al 1984) and may have contributed towards some of the increase in invasive disease observed in children in recent years.

The importance of surveillance across the entire age spectrum is emphasised by the detection of this increase in adult disease - an unexpected consequence of the widespread use of Hib vaccine. Of the eleven countries participating in the European Union Invasive Bacterial Infections Surveillance Network (EU-IBIS), only the Netherlands has noted a similar recent rise in adult Hib cases, from 8 reports in 2001 to 15 in 2002, the same number observed before vaccine introduction in 1993 (Source: National Reference Laboratory for Bacterial Meningitis, the Netherlands). Given the relative size of the two populations, this incidence is approximately half that observed in adults in the UK last

year, but similarly mirrors an increase in paediatric reports in the Netherlands (Rijkers GT et al 2003).

2.5 Impact of Hib immunisation on carriage in the UK

Literature Review

In order to understand trends in invasive Hib infection in immunised and unimmunised cohorts, a greater understanding of the vaccine's effect on Hib transmission in the UK was required. A cross sectional population based study of Hib carriage prevalence was conducted in Wales in the late 1990s, as described in Chapter 1, Section 1.5 (Figure 1.1b) (Howard AJ et al 1988). The only other study of this type undertaken in the UK took place in the 1950s and showed similar results (Dawson B et al 1952). In order to be able to monitor the effect of Hib vaccine on colonisation, the PHLS conducted a series of carriage studies in pre-school aged children in England and Wales following the introduction of Hib vaccine into the routine infant immunisation schedule in 1992. In addition, a single survey of Hib carriage in children of primary school age was conducted in Oxfordshire by the OVG in late 2001.

Outbreaks of Hib infection within nurseries were rare but well described prior to widespread vaccine use (Fleming DW et al 1985) (Osterholm MT et al 1987)(Murphy TV et al 1987)(Makintubee S et al 1987)(Marks MI 1987). The HRU was notified of an outbreak of Hib among fully immunised children in a nursery in Northamptonshire in 2002. An investigation was conducted in order to study the likelihood of Hib transmission and the role of chemoprophylaxis in this setting.

2.5.1 Carriage Studies

Methods

Personnel in six public health laboratories (Bangor, Manchester, Ipswich, Newcastle, Gloucester, Swansea) recruited children in local playgroups, nursery schools and child welfare clinics on two occasions, in June/July 1992 and 1994. A third survey was performed in Bangor and Manchester in June/July 1997, and a fourth in Oxfordshire and Gloucestershire in 2002. In a separate study of pneumococcal carriage, 103 children of the same age had swabs sent for Hib isolation in June 2002 (R George, personal communication). Children recruited in 1992 were not eligible for vaccination; children swabbed in 1994 would have been offered a single dose of Hib conjugate vaccine between 1 and 4 years of age as part of an initial 'catch-up' immunisation programme and those studied in 1997 and 2002 would have been offered routine Hib vaccine at 2, 3 and 4 months of age.

Children between 5 and 10 years old were recruited for a separate swabbing study in Oxfordshire schools in late 2001, conducted by the OVG. Seven schools took part in a variety of locations including suburban Oxford, nearby market towns and villages. The study was approved by the Oxfordshire Research Ethics Committee, the Oxfordshire Health Authority and the Local Education Authority.

Microbiological methods were essentially the same in both sets of surveys. After obtaining parental consent, throat swabs were collected and inserted into *Haemophilus* transport medium. They were then plated onto enriched Columbia Hib antiserum agar plates which were incubated at 37°C and in 5% CO_2. Due to a shortage of antiserum for the nursery studies in 1997 and 2002, some swabs were initially plated onto chocolate agar. Presumptive Hib colonies were subcultured and referred to the HRU in Oxford for serotyping, and confirmatory genotyping.

Results

The results of the nursery studies demonstrated the expected decline in Hib carriage prevalence (Table 2.5). Ninety-five percent confidence intervals for the 1992 estimate did not overlap with those from any other year. In 2002, a year of increased invasive disease incidence, combined data from separate studies in pre-school children still revealed no carriers among 384 individuals swabbed.

Four hundred and seventy-six oropharyngeal swabs were collected from primary school-aged children in Oxfordshire. Eight children [1.7% (0.07, 3.3)] were found to be carrying Hib, two carried serotype e (0.4%), and another two were colonised with serotype f (0.4%). Prevalence appeared to increase with age, although numbers were small (Table 2.6). Five of the Hib carriers attended one school, and three of these were in the same year level (Year 5), but none were related.

Table 2.5: Hib carriage prevalence, PHLS nursery studies

a) By year of collection and age

Age/Year	1992		1994		1997		2002	
1-1.99 yrs	0/17	(0%)	0/27	(0%)	0/4	(0%)	0/69	(0%)
2-2.99 yrs	9/349	(2.58%)	0/279	(0%)	0/129	(0%)	0/96	(0%)
3-3.99 yrs	30/904	(3.32%)	7/888	(0.79%)	0/229	(0%)	0/134	(0%)
4-4.99 yrs	22/261	(8.43%)	4/369	(1.08%)	0/96	(0%)	0/85	(0%)
Total	61/1531	(3.98%)	11/1563	(0.70%)	0/458	(0%)	0/384	(0%)

b) By year of collection and location (* denotes use of chocolate agar for initial plating)

Location/Year	1992		1994		1997		2002	
Bangor	9/306	(2.94%)	1/292	(0.34%)	0/191*	(0%)		
Gloucester	8/249	(3.21%)	4/222	(1.80%)			0/78*	(0%)
Ipswich	5/296	(1.69%)	1/282	(0.35%)				
Manchester	10/258	(3.88%)	2/266	(0.75%)	0/267*	(0%)		
Newcastle	27/303	(8.91%)	3/292	(1.02%)				
Oxford							0/203	(0%)
Pneumo study							0/103*	(0%)
Swansea	2/119	(1.68%)	0/209	(0.0%)				
Total	61/1531	(3.98%)	11/1563	(0.70%)	0/458	(0%)	0/384	(0%)
(95% CI)		(3.06, 5.09)		(0.35, 1.26)		(0, 0.80)		(0, 0.96)

Table 2.6: Prevalence of Hib carriage by age, Oxfordshire school study

Age	Number of Children Swabbed	Number of Hib Carriers	Carriage Prevalence
5-6 years	58	0	0
6-7 years	79	2	2.5%
7-8 years	86	0	0
8-9 years	112	1	0.9%
9-10 years	76	1	1.3%
10-11 years	65	4	6.2%
All ages	476	8	1.7%

2.5.2 Investigation of nursery Hib outbreak

Methods

The HRU was notified of an outbreak of Hib in a nursery in Wellingborough, an urban market town in the East Midlands of England (population: 70,000). 94 children, including 4 pairs of siblings, were enrolled in this centre, cared for by 21 staff. Two children of a part time staff member attended for several hours each week. Care was in four rooms divided by age, with 10 children less than 18 months, 29 aged 18-30 months, 22 aged 30-36 months and 33 older than 36 months. The younger two and the older two groups ate meals together.

Paediatricians from Kettering General Hospital alerted Northampton Health Protection Unit to two cases of invasive Hib infection in children who attended this nursery occurring within one week. The first child, aged 15 months, presented with meningitis, and the second, a 13 month old, with septic arthritis. The nursery manager assisted in notifying staff and parents of enrolled children to attend for rifampicin prophylaxis (20 mg/kg/day as a single dose for 4 days). Hib vaccination dates and batch numbers for all children were

confirmed from regional Child Health Computer records, or General Practitioners. Parents and staff gave consent to swabbing and questionnaire studies relating to Hib carriage.

Oropharyngeal cultures were obtained using described microbiological methods (Barbour ML et al 1993). Both tonsillar fossae were sampled using a cotton tipped wooden swab, which was immediately placed into tryptone soy broth enriched with X and V factors. Swabs were plated onto enriched Columbia antiserum agar plates 2-6 hours later using a standardised method with radial streaking of a central 5 μl inoculum, and a heavier peripheral inoculum. They were then incubated at 37°C and in 5% CO_2 for 48 hours. Colonies exhibiting iridescent haloes were subcultured and identified as type b by serotyping and PCR, through the PHLS HRU. Antibiotic sensitivities were demonstrated by standard disc diffusion methods, and minimal inhibitory concentrations (MICs) determined using the Etest® strip system (AB Biodisk, Sweden). In order to further characterise the relatedness of strains, comparative restriction endonuclease digests of chromosomal DNA were performed using the enzymes Mfel and Pstl.

The rooms in which children were cared for and the number of hours per week in nursery were derived from attendance records. Parents were asked to complete a questionnaire detailing exposure to factors known to influence the likelihood of Hib carriage, including crowding and smokers in the household (increased risk) and breast feeding (decreased risk) (Cochi SL et al 1986).

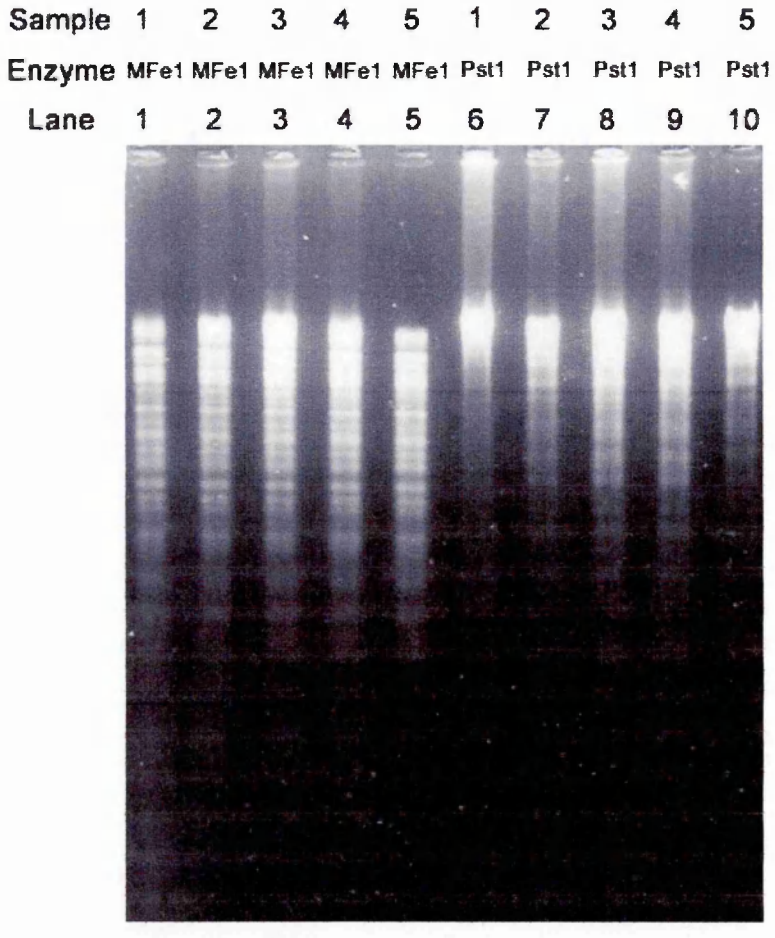
Results

Ninety-three children in the nursery were fully immunised against Hib. One was only old enough to have received two doses. Eighty-nine of 92 children without Hib disease, both index cases, and all staff members received Rifampicin prophylaxis (uptake 97.4%). One parent refused the medication for her child and a further two did not attend. Twins with intercurrent illness received the medication at home.

Swabs were collected from 87 of the 88 available healthy children (one refused), all 21 staff and 2 children of a staff member. Two Hib carriers were identified, one child and one staff member, from the same room as the index cases. One child in another room carried a serotype f strain. The four Hib isolates were resistant to Trimethoprim, with the same MIC (1.5 μg/ml), and showed a similar migration pattern on gel electrophoresis (Figure 2.12). One month later, 7 of the 10 children in this room were re-swabbed, with their 3 carers. Two (one an index case) had left the nursery, and one was on holiday. No Hib strains were isolated on this occasion.

Of the three children in the under 18 months' room with evidence of Hib exposure, two attended nursery for more than 40 hours per week, compared with one of 7 non-carriers. One colonised child had been breastfed (33%), compared with 6 of 7 not colonised (86%). Only the child with asymptomatic carriage shared a bedroom at home, and the only child with a smoker in the household was not a carrier. All of those who were colonised, and 5 of the 7 who were not, reported recent upper respiratory infection, but none had received antibiotics.

Figure 2.12: Electrophoretic gel migration patterns of isolates from Hib nursery outbreak, following digestion with the restriction endonucleases MFe1 and Pst1. Isolates 1 and 2 are the invasive disease strains, 3 and 4 the carriage strains, and 5 is the reference strain RM7004.



Conclusions – carriage

In the PHLS studies, the prevalence of asymptomatic Hib colonisation decreased in pre-school children within a few years of introduction of the Hib vaccine programme. No resurgence of carriage was seen in later time periods when invasive disease rates were rising, with no carriers detected in the last two surveys (McVernon J et al 2004b).

Ongoing Hib colonisation among older children was evidenced by the study in Oxfordshire schools. While the overall carriage prevalence was 1.7%, clustering of isolates was observed, with multiple carriers detected in a single school. This study provided clear evidence of ongoing community transmission of Hib in an age group previously postulated to introduce the organism into family settings, thereby exposing infants to risk (Ounsted C 1951).

Within a single room in the nursery studied where an outbreak of Hib disease had occurred, 3 of 10 (30%) children and 1 of 3 (33%) carers acquired Hib. Such figures are comparable to the observed carriage prevalence in unvaccinated nursery contacts of children with invasive disease (Prober CG et al 1982) (Li KI et al 1986). Infants immunised with conjugate Hib vaccine show delayed acquisition of carriage (Barbour ML et al 1995), but carry at the population rate by pre school age (Barbour ML et al 1993). Transmission pressure is thus capable of overwhelming the vaccine's effect and must be appreciable in a nursery setting where children spend long hours together.

In an exploratory analysis of other risk factors for carriage, 6 of 7 children who did not carry Hib had been breastfed, compared with only 1 of 3 who did acquire the organism. A

history of breastfeeding has previously been found to protect infants under one year against carriage, with an odds ratio of 0.3 (0.1, 0.8) (Cochi SL et al 1986).

Rifampicin prophylaxis is recommended for children recovering from Hib disease and their household contacts based on studies from the pre-vaccine era (Cartwright KA et al 1991). Reacquisition of the same or novel strains within 10-30 days of the course of antibiotic is possible even when compliance is high (Glode MP et al 1985). None of the children and staff swabbed for a second time in our study, three of whom had previously been exposed to Hib, showed evidence of reacquisition.

An outbreak of invasive Hib infection within fully vaccinated children in a day care nursery is a rare but concerning event. Rifampicin was used effectively in this instance to block transmission of the organism. The risk of occurrence of a secondary case cannot be estimated from this single report (McVernon J et al 2004a).

2.6 Impact of Hib immunisation on population immunity to Hib in the UK

Literature Review

The acquisition of natural immunity to Hib in unvaccinated populations was thoroughly described in Section 1.9 of Chapter 1. Briefly, passive immunity was provided by maternal antibody in the first few months of life. This waned, to leave infants without measurable serum activity against Hib over the next 2-3 years (Fothergill LD et al 1933). Anti-PRP titres began to rise after the third birthday, becoming stable in adult life (Kayhty H et al 1983).

Changing population immunity to Hib in the UK following vaccine introduction was studied using two data sources. In an initial exploratory analysis, reports of Hib antibody concentrations measured at the routine immunology laboratory of the Churchill Hospital, Oxford, were compared over the years 1995 to 2001. This hypothesis generating data led to a formal study conducted by the PHLS using reference serum stored in their seroepidemiology unit (Trotter CL et al 2003b).

2.6.1 Hib antibody measurements, Churchill Hospital Oxford, 1995-2001

Methods

Anonymised laboratory reports were obtained from 14,000 Hib antibody requests referred to the Churchill Hospital routine Immunology Laboratory, Oxford between 1995 and 2001. No information was available regarding the indications for performing the measurement. Samples were taken from individuals ranging in age from birth to 80 years. Assays were conducted using the standardised HbO-HA ELISA developed by the Centres for Disease Control, Atlanta (Phipps DC et al 1990). Antibody titres were log transformed in order to approximate a normal distribution, and geometric mean concentrations calculated with 95% confidence intervals to allow between group comparisons.

Results

Distribution of ages at serum collection by year of measurement is shown in Table 2.7. Geometric mean antibody concentrations by age group and year of serum collection are detailed in Table 2.8. Results are represented graphically for the years 1995, 1997 and 2001 in Figure 2.13. The intervening years' results are omitted from the figure for the sake of clarity. A decline in Hib antibody titres was observed when 1995 was compared with subsequent years. Age groups most affected included children aged 3 to 5 years,

teenagers, and the elderly (over 50 years). There was also a suggestion of waning maternal immunity, with a reduction in antibody concentrations in infants aged 0-6 months. Because of the nature of the data represented, it was uncertain whether this apparent reduction in immunity could have resulted from changes in the laboratory assay used over time.

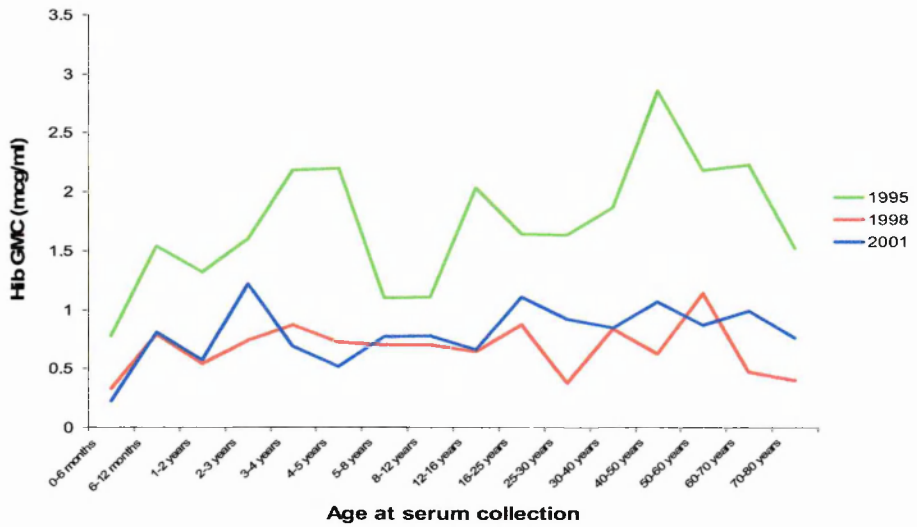
Table 2.7: Age of donor and year of serum collection, Hib antibody reports, Churchill Hospital Oxford, 1995-2001

Age	1995	1996	1997	1998	1999	2000	2001	Total
0-6 months	103	121	109	121	134	137	137	862
6-12 months	152	144	137	161	129	169	189	1081
1-2 years	262	249	184	204	223	213	246	1581
2-3 years	141	156	121	159	185	180	180	1122
3-4 years	85	123	123	132	111	144	143	861
4-5 years	80	118	134	119	102	130	100	783
5-8 years	142	176	141	218	226	194	265	1362
8-12 years	101	130	125	133	140	167	168	964
12-16 years	84	116	85	99	93	109	136	722
16-25 years	73	97	90	79	78	94	118	629
25-30 years	65	97	38	35	57	54	62	408
30-40 years	143	197	106	120	112	143	203	1024
40-50 years	121	149	76	36	71	99	150	702
50-60 years	107	129	78	65	67	114	213	773
60-70 years	151	122	63	50	71	99	173	729
70-80 years	72	109	46	48	29	62	130	496
Total	1882	2233	1656	1779	1828	2108	2613	14099

Table 2.8: Geometric mean concentrations of anti-PRP antibody by age and year of serum collection, Churchill Hospital Oxford, 1995-2001 (95% confidence intervals)

Age	Year of Serum Collection						
	1995	1996	1997	1998	1999	2000	2001
0-6 months	0.78 (0.57, 1.07)	0.70 (0.52, 0.95)	0.34 (0.25, 0.46)	0.33 (0.24, 0.45)	0.24 (0.19, 0.31)	0.36 (0.28, 0.47)	0.23 (0.18, 0.29)
6-12 months	1.54 (1.17, 2.04)	1.27 (0.96, 1.68)	0.80 (0.61, 1.06)	0.79 (0.60, 1.04)	0.72 (0.54, 0.96)	0.72 (0.57, 0.92)	0.81 (0.64, 1.02)
1-2 years	1.32 (1.05, 1.67)	1.14 (0.93, 1.40)	0.74 (0.58, 0.96)	0.54 (0.42, 0.68)	0.68 (0.55, 0.85)	0.67 (0.54, 0.84)	0.57 (0.46, 0.71)
2-3 years	1.60 (1.12, 2.28)	1.68 (1.28, 2.22)	0.76 (0.53, 1.10)	0.74 (0.54, 1.00)	0.65 (0.50, 0.85)	0.58 (0.44, 0.76)	1.22 (0.89, 1.67)
3-4 years	2.18 (1.38, 3.44)	1.44 (1.05, 1.97)	0.63 (0.44, 0.89)	0.87 (0.63, 1.21)	0.84 (0.60, 1.18)	1.14 (0.79, 1.66)	0.69 (0.50, 0.94)
4-5 years	2.20 (1.45, 3.36)	1.64 (1.23, 2.20)	0.60 (0.44, 0.81)	0.72 (0.52, 1.00)	0.72 (0.49, 1.06)	0.66 (0.47, 0.92)	0.52 (0.35, 0.78)
5-8 years	1.10 (0.79, 1.54)	1.17 (0.92, 1.50)	1.17 (0.89, 1.54)	0.70 (0.55, 0.88)	0.73 (0.58, 0.91)	0.68 (0.53, 0.88)	0.77 (0.61, 0.97)
8-12 years	1.11 (0.75, 1.64)	1.22 (0.92, 1.63)	0.65 (0.47, 0.90)	0.70 (0.52, 0.95)	0.81 (0.61, 1.08)	1.01 (0.78, 1.30)	0.78 (0.60, 1.02)
12-16 years	2.03 (1.14, 2.92)	1.44 (1.06, 1.96)	1.02 (0.72, 1.45)	0.64 (0.46, 0.90)	0.83 (0.58, 1.19)	0.53 (0.38, 0.73)	0.66 (0.49, 0.90)
16-25 years	1.64 (1.05, 2.56)	1.20 (0.87, 1.65)	0.95 (0.67, 1.35)	0.87 (0.57, 1.31)	0.96 (0.65, 1.42)	1.03 (0.76, 1.41)	1.11 (0.83, 1.48)
25-30 years	1.63 (1.12, 2.36)	1.13 (0.80, 1.60)	1.08 (0.62, 1.88)	0.38 (0.23, 0.64)	0.55 (0.36, 0.84)	0.60 (0.39, 0.95)	0.92 (0.58, 1.45)
30-40 years	1.87 (1.41, 2.46)	1.18 (0.95, 1.47)	0.77 (0.57, 1.06)	0.84 (0.62, 1.14)	0.71 (0.51, 0.98)	0.73 (0.56, 0.95)	0.85 (0.67, 1.08)
40-50 years	2.86 (2.08, 3.93)	1.20 (0.91, 1.60)	1.03 (0.70, 1.51)	0.63 (0.37, 1.09)	0.86 (0.57, 1.31)	1.27 (0.92, 1.76)	1.07 (0.81, 1.42)
50-60 years	2.18 (1.55, 3.06)	1.19 (0.91, 1.56)	0.75 (0.50, 1.12)	1.14 (0.71, 1.83)	0.91 (0.59, 1.40)	0.70 (0.52, 0.94)	0.87 (0.68, 1.10)
60-70 years	2.23 (1.69, 2.94)	1.24 (0.91, 1.68)	0.83 (0.56, 1.25)	0.47 (0.29, 0.76)	0.51 (0.35, 0.75)	0.52 (0.37, 0.71)	0.99 (0.74, 1.32)
70-80 years	1.52 (1.06, 2.17)	1.14 (0.82, 1.58)	0.71 (0.44, 1.16)	0.40 (0.26, 0.63)	0.71 (0.36, 1.42)	0.44 (0.29, 0.67)	0.76 (0.55, 1.06)

Figure 2.13: Geometric mean concentrations (GMCs) of Hib antibody by age group and year, Churchill Hospital Oxford 1995-2001



2.6.2 HPA Seroepidemiology study

Methods

The impact of Hib vaccine introduction on population immunity was formally studied using sera stored by the HPA Seroepidemiology Unit. Serum left over from diagnostic specimens is collected anonymously each year from a range of laboratories across England for this purpose (Osborne K et al 2000). Testing for antibodies to PRP was conducted at the HPA, Porton Down, using the same HbO-HA ELISA described above (Phipps DC et al 1990). Sera from children under the age of 16 in 1990/1991, 1994, 1997 and 2000 were selected to represent pre- and post-vaccine periods. In addition, samples collected from adults aged 30-39 years were also tested from the same time points.

Antibody concentrations by age and year of collection were reported as medians, due to the highly skewed nature of the data. The significance of differences between groups was assessed using the Kruskal-Wallis test, in STATA 7.0. Titres were also represented in

relation to the previously described thresholds of 0.15 and 1.0 μg/ml, thought to correlate with protection against Hib infection (Kayhty H et al 1983).

Results

These data were collated by Dr Caroline Trotter of the Statistics, Modelling and Economics Department, HPA, Colindale, who authored the paper describing the paediatric component of this study (Trotter CL et al 2003b). Figures and tables below are taken from the manuscript.

Striking changes in levels of immunity to Hib were observed over the period of the study (Figure 2.14). With the introduction of immunisation, a significant increase in Hib antibody concentrations was observed in one-year old infants, which persisted in all subsequent time points ($p<0.0001$ for all comparisons with 1990/1991). Most evident was the impact of catch-up immunisation on Hib antibody concentrations in children aged between 3 and 4 years in 1994. Children of the same age in later years had much lower titres, consistent with rapid waning of immunity following primary infant immunisation. A corresponding increase in the proportion of toddlers without detectable serum antibody against PRP was observed (Figure 2.15).

Antibody levels to Hib in 30-39 year old English adults, collected at four time points over the period, showed a trend in keeping with the numbers of reports of invasive disease (Figure 2.8). A significant fall in median Hib antibody titres was noted from 1.29 μg/ml (0.90, 1.64) in 1991 to 0.70 μg/ml (0.57, 0.89) in 1994 ($p=0.006$) with a further non-significant decrease to 0.53 μg/ml (0.35, 0.73) in 1997. A small non-significant increase to 0.69 μg/ml (0.52, 0.95) was seen in 2000 ($p=0.197$).

Figure 2.14: Median Hib antibody concentrations, by age and year (from Trotter CL et al 2003b)

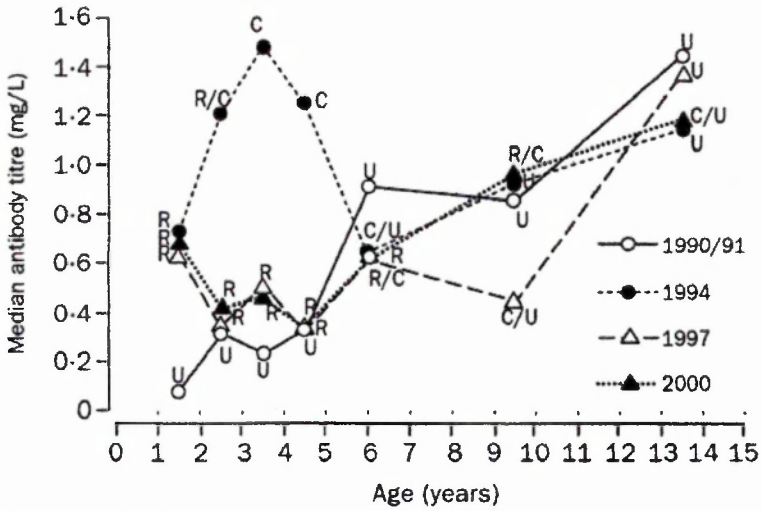
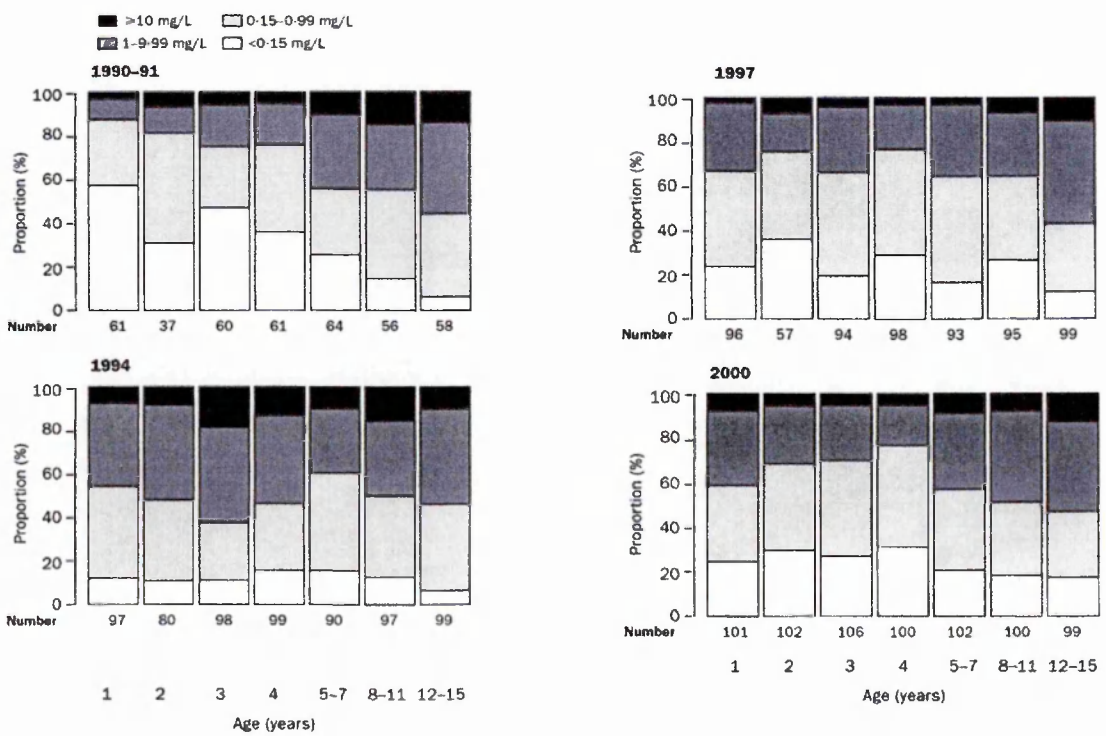


Figure 1: **Median Hib antibody titres**
(R=routinely vaccinated group, C=catch-up group, U=unvaccinated cohort).

Figure 2.15: Hib antibody concentrations in relation to the thresholds 0.15, 1.0 and 10 μg/ml, by age and year of collection (from Trotter CL et al 2003b)



Conclusions – population immunity

Infant immunisation has resulted in the induction of antibody titres deemed protective in the age group at greatest risk of serious Hib infections, namely infants less than 24 months of age. These antibodies wane rapidly, within 2 to 3 years, in the absence of a booster dose. The catch-up immunisation campaign that accompanied vaccine introduction in 1992 and 1993 resulted in very high levels of immunity throughout the first five years of life, seen in both the Oxfordshire and HPA datasets, which have not been subsequently observed in cohorts immunised in infancy.

Adults and older children who had never been immunised experienced a decline in overall levels of Hib antibody within two years of vaccine introduction. This reduction in immunity was initially temporally associated with falling disease incidence, and may have contributed to a recent resurgence in invasive Hib infections in adults.

2.7 Conclusion

Immunisation against Hib was introduced in the UK in October 1992 as an accelerated primary schedule at 2, 3 and 4 months of age without a booster dose. Vaccine introduction was supplemented over the first year of use by a national catch-up immunisation campaign involving children under the age of four years. Coverage of the three dose primary course by the age of one year remained greater than 90% over the next ten years. Hib vaccines used in the UK have changed over time, from single antigen vaccines to combinations with diphtheria, tetanus and whole cell pertussis (DTwP-Hib). Due to problems with vaccine supply, a similar preparation containing acellular pertussis (DTaP-Hib) was used in 2000/2001.

The UK's immunisation strategy was very effective in inducing a rapid reduction in the incidence of disease among vaccinated children. An associated decrease in oropharyngeal Hib carriage was responsible for a 'herd immune' effect. As a result, the incidence of Hib disease in cohorts too young or old to be immunised also declined. A slight increase in the average age at Hib presentation, from the second to the third year of life, reflected high protective efficacy in the youngest infants. No other significant changes in the clinical presentation of Hib disease occurred, in vaccinated or unvaccinated individuals. In addition, no evidence of a shift in disease to other capsular serotypes was observed.

Despite this initial success, a steady increase in the incidence of invasive Hib infections ascertained primarily through laboratory reporting was observed in children and adults in England and Wales from 1999 onwards. Most of these children had been fully immunised. Similar trends in hospital admissions for epiglottitis in childhood were seen, confirming the microbiology data.

The rise in disease incidence was not accompanied by an evident increase in Hib colonisation in children of pre-school age. Ongoing Hib transmission in the community was demonstrated by detection of carriage in children attending primary schools, and through experience of an outbreak of disease among vaccinated infants and toddlers in a day care centre. Adults in age groups most commonly exposed to children in household settings experienced higher rates of invasive infection than the very elderly.

Study of changes in population immunity to Hib resulting from vaccine introduction added to our understanding of invasive disease trends. Levels of specific anti-PRP antibody in children under two years of age rose following introduction of infant immunisation. This

observation correlated with the greatest reduction in invasive disease within any age group. The initial Hib catch-up immunisation campaign was associated with induction of very high antibody titres in toddlers, which persisted throughout the first five years of life. Subsequent cohorts of children, immunised only in infancy, had much lower levels of antibody to Hib from three years of age onwards as antibody concentrations waned rapidly following the primary course. Children born after the catch-up period showed a progressive reduction over time in the duration of antibody protection associated with immunisation, most marked in 2000. The beneficial effects of the catch-up campaign on immunity may have enhanced the apparent impact of the infant primary schedule. The other change temporally associated with reduced vaccine protection in 2000 was use of DTaP-Hib vaccines.

A significant fall in the median anti-PRP antibody concentration in English adults occurred only two years after Hib vaccine's implementation. The interruption of Hib transmission observed in children may be expected to have reduced opportunities for natural boosting of immunity in all age groups. This would suggest that recurrent colonisation could be required to maintain high levels of serum immunity. The subsequent creation of a susceptible pool of adults might have contributed to a resurgence of disease.

Chapter 3 – Factors associated with increasing Hib incidence in epidemiological analyses

3.0 Introduction

In examining the possible reasons for the increase in Hib incidence in the UK, it must be borne in mind that the application of vaccines in whole populations is much more complex than the controlled environment of a Phase III efficacy trial. Vaccine schedules evolve as new public health priorities and products emerge on the scene. With respect to the Hib vaccines themselves, the formulation and availability of manufacturers' products has changed over time, with consequences for immunogenicity.

A number of host and environmental variables were associated with increased risk of invasive Hib infection before immunisation was available (Section 1.8 of Chapter 1). If vaccine efficacy was reduced in susceptible individuals, niches for ongoing transmission and disease might occur. Sociological changes leading to an expansion of 'at-risk' groups in the population may be expected to be associated with changes in epidemiology over time. Further, identification of clinical and immunological risk factors contributing to vaccine failure would be desirable in order to offer such children additional protection through extra doses of vaccine or other measures. The assessment of clinical, social and environmental factors correlating with disease risk is essential in order to appreciate the contribution of non-vaccine related variables to disease trends.

Unanticipated effects of vaccine introduction on transmission, while beneficial in the short term, may have far reaching effects on boosting of natural immunity within a population. Such an impact may not be apparent in an efficacy trial, where vaccinated individuals move within a wider unimmunised community. The resulting creation of new pools of susceptible individuals may open up opportunities for transfer of infection and increased rates of progression to invasive disease.

These issues have been addressed in part in the course of a number of studies conducted by the HPA, OVG and BPSU over the past ten years. Findings to date will be outlined in the following chapter.

3.1 Vaccine factors associated with changing Hib epidemiology in the UK, 1993-2002

Literature Review

Catch-up immunisation of children up to 4 years of age with the HbOC conjugate vaccine was an integral part of the UK's Hib immunisation strategy over the first year of introduction. As described in Section 2.3 of Chapter 2, a range of Hib conjugate vaccine formulations have subsequently been licensed for use in the UK's infant schedule over the past 10 years. PRP-T was initially administered as a single antigen for routine infant immunisation. Combination DTwP-Hib vaccines were widely used from 1996 onwards, with a switch to a single manufacturer's DTaP-Hib vaccine from late 2000 to mid 2001.

Antibody titres following a single dose of Hib conjugate vaccine increased with age at administration (Madore D et al 1990a, 1990b) (Rothstein EP et al 1991). In

infancy, the nature and schedule of vaccine also impacted on peak anti-PRP concentrations achieved (Vidor E et al 2001). While there was no consistent evidence that combination with DTwP had any effect on immunogenicity of the Hib component (Vidor E et al 2001), DTaP caused a significant reduction in immunity to Hib when administered in the same syringe as PRP-T (Bell F et al 1998) (Schmitt HJ et al 1998). The reduction in Hib antibody titres was most striking in an accelerated schedule such as that used in the UK (Vidor E et al 2001), and was more noticeable with each additional dose of DTaP-Hib given as part of the primary course (Daum RS et al 2001). Based on demonstration of priming for immunologic memory following DTaP-Hib in infancy (Zepp F et al 1997) (Goldblatt D et al 1999) (Poolman J et al 2001), the clinical relevance of these lower antibody concentrations to protection against disease was questioned (Eskola J et al 1999) (Poolman J et al 2001).

Early estimates of vaccine impact reported from the UK compared rates of Hib disease before and after 1992, thereby measuring both direct and indirect (herd immunity) effects of immunisation. Reported vaccine efficacy over the first five years of life as assessed by these means was 97.6% (Heath PT et al 2000a). Given the changing epidemiology of Hib in the years following conjugate vaccine introduction, a further analysis of vaccine efficacy was conducted by the HPA in order to see whether different vaccines or schedules were temporally associated with altered protection. As both vaccinated and unvaccinated children were subject to the same degree of indirect protection conferred by herd immunity, contemporaneous incidence rates were compared between the two groups to allow measurement of direct vaccine efficacy.

In a further study, the possible contribution of DTaP-Hib to a resurgence in disease was examined by comparing vaccines received by Hib vaccine failures (cases) with those received by fully immunised children without disease (controls).

3.1.1 Analysis of vaccine efficacy

Methods

The HPA study of direct vaccine efficacy has been published (Ramsay ME et al 2003). Methods of case ascertainment for invasive Hib infection and confirmation of vaccination status were as described earlier in Section 2.4. Full protection was assumed from 7 days after the completion of 3 doses of vaccine before 13 months, or a single dose after this age. Partially vaccinated children were excluded from the analysis. Coverage data for routine and catch-up immunisation were derived from sources used previously (http://www.hpa.org.uk/cdr/archive04/immunisation04.htm) (O'Brien H 1994).

Effectiveness was calculated by the screening method, which is described in the following equation:

$$\textit{Vaccine effectiveness} = 1-[PCV(1-PPV)]/[[(1-PCV)PPV]$$

where PCV is the proportion of the cases who are vaccinated and PPV is the proportion of the population vaccinated, or vaccine coverage. Direct vaccine protection was assessed by period of birth and time since scheduled vaccination using univariate and multivariate logistic regression models. The statistical analysis

for this study was conducted by Nick Andrews of the HPA's Statistics, Modelling and Economics Department. Full details of the statistical methods employed are outlined in the published paper (Ramsay ME et al 2003).

Results

Table 3.0 is derived from Table 3 of Ramsay ME et al 2003.

Table 3.0: Summary of vaccine effectiveness estimates (%) with 95% confidence intervals by year of birth and time since vaccination.

Vaccination schedule, period of birth	**Efficacy overall (95% CI)**	**Efficacy <2 yrs (95% CI)**	**Efficacy >2 yrs (95% CI)**
Catch-up	*87.1*	*97.1*	*71.7*
Jan 1989-Jul 1992	*(65.5, 95.2)*	*(75.4, 99.7)*	*(3.2, 91.7)*
Infant	*49.4*	*60.9*	*27.3*
Aug 1992-Dec 2001	*(31.8, 64.1)*	*(42.0, 73.6)*	*(-28.8, 59.0)*
Infant	48.6	74.5	-55.9
Aug 1992-Dec 1993	(-31.6, 9.9)	(25.5, 91.9)	(-1046, 78.8)
Infant	75.1	67.4	79.8
Jan 1994-Dec 1995	(50.3, 87.5)	(4.8, 88.8)	(51.7, 91.5)
Infant	65.4	88.0	29.9
Jan 1996-Dec 1997	(26.5, 83.7)	(64.2, 96.0)	(-125, 78.1)
Infant	42.0	72.8	-17.0
Jan 1998-Dec 1999	(-16.6, 71.2)	(32.6, 89.0)	(-272, 63.2)
Infant	25.8	35.6	$-\infty$
Jan 2000-Dec 2002	(-36.3, 59.6)	(-17.8, 64.8)	(∞, 72.0)
Total	**56.7**	**66.1**	**37.3**
Either schedule	**(42.5, 67.4)**	**(51.2, 76.4)**	**(-3.1, 61.9)**
Jan 1989-Dec 2001			

In brief, the overall estimate of vaccine efficacy in the UK was 56.7% (42.5, 67.4), which was surprisingly low. Even allowing for 2% underestimation of vaccine coverage, efficacy only rose to 71.8%. Children vaccinated over 1 year of age during the catch-up campaign [87.1% (65.5, 95.2)] were much better protected than those immunised in infancy [49.4% (31.8, 64.1)] (Table 3.0 and Figure 3.0). Vaccine protection waned rapidly, falling sharply beyond two years of immunisation (Table 3.0 and Figure 3.0). A decline in short term protection conferred by infant immunisation was observed in cohorts of children born in 2000 and 2001 when compared with those born in other years (Table 3.0 and Figure 3.1).

3.1.2 Analysis of vaccines administered to cases and controls

Methods

DTaP-Hib was distributed in the UK between January 2000 and October 2001 (Section 2.3). Hib cases born between 01/10/1999 and 31/06/2001 were thus selected for analysis. Hib immunisation dates and batch numbers were confirmed with general practitioners or child health computer records. Date of birth-matched controls were chosen from anonymised immunisation data downloaded from a single child health system covering children resident in the South-East of England. Vaccines were identified from batch numbers, using information supplied by the manufacturers. In the few instances where there was any uncertainty regarding the formulation of one manufacturer's product, batches were categorised as DTwP-Hib for cases and DTaP–Hib for controls. Conditional logistic regression was used to compare the number of doses of the full primary immunisation series given as DTaP-Hib between each case and their matched controls. The statistical analysis was conducted by Nick Andrews of the HPA's Statistics, Modelling and Economics Department (McVernon J et al 2003a).

Figure 3.0: Vaccine efficacy by schedule and time since vaccination *(95% CIs)*

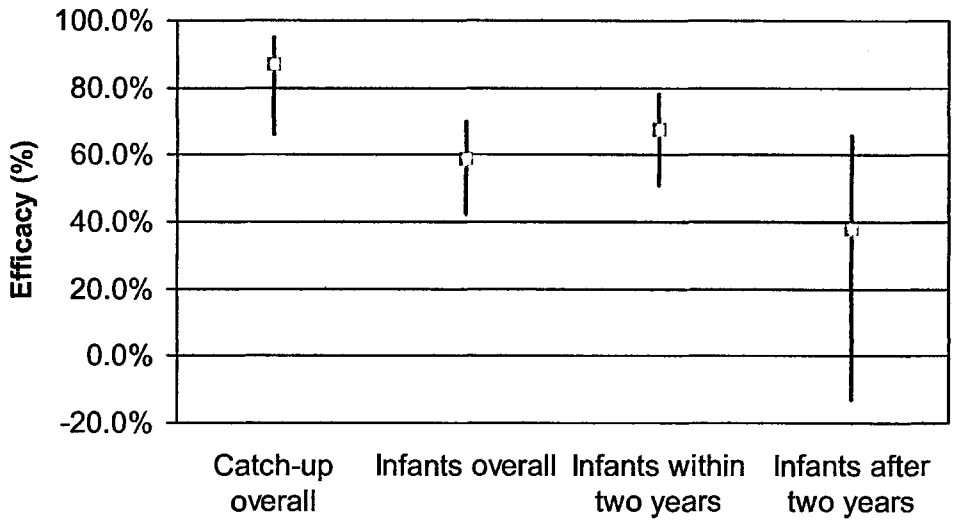
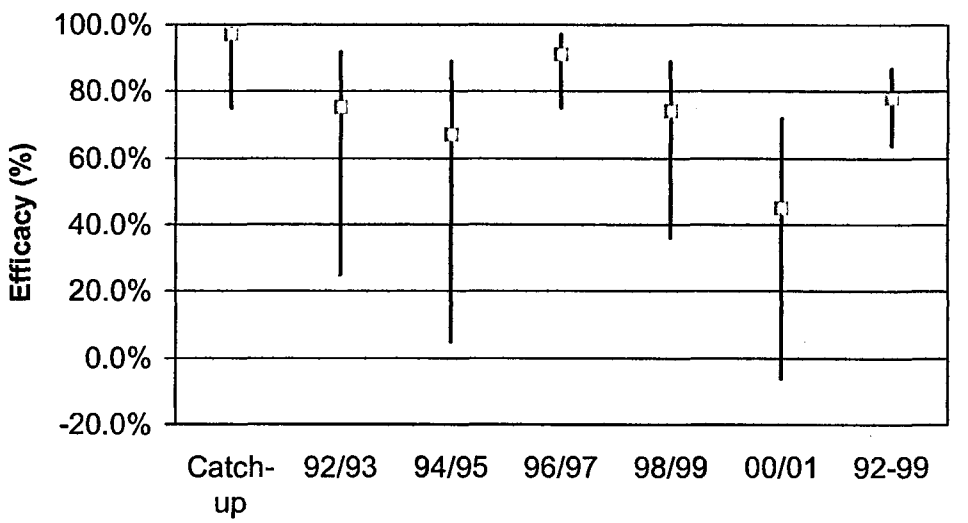


Figure 3.1: Short term vaccine efficacy by birth cohort (95% CIs)



Results

One hundred and thirty cases who had received three doses of vaccine before disease were identified. Complete batch number information was unavailable for 19 (15%), leaving 111 for study. 3,834 (64%) of an initial control dataset of 5,952 fully vaccinated children had a full set of valid batch numbers recorded. Each case was matched by date of birth (DOB) with a median of 39 controls (range 4 to 60).

Age at the third dose of vaccine differed between groups, with 18% (701/3,834) of controls and 9% (10/111) of cases completing the course over 160 days of age. As this difference may have influenced access to the DTaP-Hib combination, a further analysis was performed in which controls were restricted to include only those who completed vaccination in the same time frame as their DOB matched index case. Age at completion was stratified as follows: <120 days, 120-159 days and ≥160 days.

Results of the conditional logistic regression analyses are shown in Table 3.1. Significantly more cases than controls had received three doses of DTaP-Hib during their primary vaccination schedule compared with one or no doses. A significant trend for increasing risk of vaccine failure with each additional dose of the aP combination was also observed. No effect of age at third dose was observed (McVernon J et al 2003a).

A. Analysis of Cases and All Age-matched Controls				
	Number of DTaP-Hib doses received			
	0	1	2	3
Case	24	15	17	55
Controls	1223	783	612	1216
Conditional OR, 95% CI	1	1.11 (0.56, 2.22)	2.21 (1.07, 4.55)	6.35 (3.06, 13.18)
Trend per dose: 1.87 (1.46, 2.40) p<0.00001				
B. Age Stratified Analysis				
	Number of DTaP-Hib doses received			
	0	1	2	3
Case	24	15	17	55
Controls	519	309	248	576
Conditional OR, 95% CI	1	1.22 (0.59, 2.53)	2.60 (1.19, 5.65)	7.79 (3.52, 17.25)
Trend per dose: 1.99 (1.52, 2.59) p<0.00001				

Table 3.1: Doses of DTaP-Hib received by cases and DOB matched controls (from McVernon J et al 2003a)

Discussion – vaccine factors

Changes over time in the levels of specific immunity to Hib observed in British children were noted in Section 2.6 of Chapter 2. A marked positive impact of catch-up immunisation on peak antibody titres achieved and persistence throughout the period of risk was observed (Trotter CL et al 2003). The analysis of vaccine efficacy has demonstrated that such an immunogenicity profile was associated with more effective protection against invasive Hib disease than primary infant immunisation. Further, in keeping with the observed rapid waning of antibody titres following the primary course, efficacy declined sharply in the two years following vaccination in infancy (Ramsay ME et al 2003).

An additional reduction in vaccine effect was noted in 2000/2001, when compared with infants born in other years (Ramsay ME et al 2003). It had previously been observed that the age of presentation with invasive disease declined in vaccinated children born in those years (Section 2.4.2, Figure 2.7), implying a shorter duration of vaccine protection. As had also been noted in Section 2.3 of Chapter 2, these were years during which a single manufacturer's DTaP-Hib combination vaccine was distributed in the UK, due to a shortage of DTwP-Hib. The hypothesis that the reduced immunogenicity of DTaP-Hib vaccines may have resulted in reduced protection against infection was tested in a matched case-control study design. An increased risk of Hib vaccine failure was noted in fully immunised children who had received 2 or 3 doses of their primary course as DTaP-Hib, compared with 3 doses of another Hib vaccine (McVernon J et al 2003a). Thus, the clinical importance of the lower antibody concentrations was confirmed when this vaccine was used in an accelerated primary schedule without a booster dose.

3.2 Host and environmental factors associated with ongoing Hib disease in the UK, 1993-2002

Literature review

A range of host and environmental factors associated with increased individual risk of invasive Hib infection before introduction of widespread immunisation were outlined in Section 1.8 of Chapter 1. The most consistent findings in all analyses were a history of known immunodeficiency in the host, and a range of variables associated with increased exposure to other children in close settings.

As part of a prospective surveillance study of Hi disease, the OVG initiated a questionnaire-based study asking clinicians to identify pre-existing conditions that may be associated with heightened susceptibility to infection in children experiencing Hib vaccine failure. In addition, acute and convalescent serum specimens were collected, where possible, for measurement of immunoglobulins and specific antibody to PRP. Interim results were reported in 1997 (Booy R et al 1997) and 2000 (Heath PT et al 2000b).

Of children immunised in the first year of life who presented with vaccine failure, 20% were stated to have an underlying clinical risk factor (prematurity, malignancy, dysmorphic syndrome, Down's syndrome, neutropaenia) (Heath PT et al 2000b). A subsequent analysis of the contribution of prematurity to vaccine failure found no significant increase in risk in premature infants compared with those born at term [OR 1.5 (95% CI 0.9, 2.6)] (Heath PT et al 2003). An earlier age at onset of disease was observed in those with a history of preterm delivery, in keeping with the reduced immunogenicity of conjugate vaccines in such infants (Heath PT et al 2003).

Immunologic testing revealed that 30% of UK Hib vaccine failures showed some abnormality of immunoglobulin classes or IgG subclasses compared with age-specific reference values (Heath PT et al 2000b). These findings were consistent with other studies of conjugate vaccine failures in the United States and the Netherlands (Holmes SJ et al 1991, 1992) (Breukels MA et al 2001). While a previous association had been reported between immunoglobulin subclass deficiency and poor specific antibody responses to polysaccharide antigens, these findings were of uncertain diagnostic or prognostic significance (Shackelford PG et al 1990).

Despite the 'immunodeficiency' thus represented, most children who developed invasive Hib infection following conjugate vaccine mounted an antibody response to infection that was greater than expected for age (Holmes SJ et al 1991, 1992) (Breukels MA et al 2001). In those who did not, a booster dose almost always elicited antibody concentrations deemed 'protective' (Holmes SJ et al 1991) (Heath PT et al 2000b) (Breukels MA et al 2001). The HibId-1 idiotype was equally predominant in both vaccinated and unvaccinated children developing Hib disease (Holmes SJ et al 1991). One study reported reduced convalescent antibody concentrations and low avidity in children experiencing Hib conjugate vaccine failure, but these conclusions were questionable due to small numbers of cases and the use of inappropriate controls (Breukels MA et al 2002).

Only one study, conducted in the United States, has addressed social and environmental risk factors for invasive Hib infection after vaccine introduction (Jafari HS et al 1999). The vast majority of cases in this series were not age appropriately immunised. Social deprivation was a major risk factor for undervaccination, highlighting difficulty of access to routine medical care for the economically disadvantaged in the US (Jafari HS

et al 1999). In the UK, immunisation is provided as a free publicly funded service to all, resulting in consistently high immunisation coverage (Figure 2.1). One may thus expect different variables to be associated with ongoing Hib infections in the two populations.

Results from the last BPSU report on invasive Hi infection before it was withdrawn from the orange card reporting scheme at the end of 2000 are shown, thus updating findings of the OVG study of clinical risk factors. An analysis of antibody responses in Hib vaccine failures using serum collected from this study, compared with historical unvaccinated controls, is also described.

In response to the increase in cases from 1999 onwards, the HPA conducted a national case-control study of children presenting with invasive Hib infection between January 1998 and January 2003. Associations with a range of clinical variables addressed by the cohort study were reassessed within this new design. In addition, information was sought on social and environmental factors known to be correlated with altered risk of disease in the pre-vaccine era.

3.2.1 Study of clinical and immunological risk factors associated with Hib vaccine failure

Methods

Case ascertainment for invasive Hi infection has already been described. Clinicians caring for children experiencing Hib vaccine failure were sent a postal questionnaire enquiring after the child's vaccination status and asking them whether the child had a known underlying illness or immunodeficiency which may have predisposed them to

their infection. In addition, they were asked to tick any of a relevant series of boxes if the child had a history of prematurity, malignancy, dysmorphic syndrome, Down's syndrome, chromosomal anomaly or neutropaenia. Written parental consent to release of this information was required after the study's reapproval by the South East Multi-Centre Research Ethics Committee in 2001, resulting in a marked reduction in the number of complete reports obtained (Dr M Slack, personal communication).

Results

By the BPSU study's completion at the end of October 2000, 719 reports that met the case criteria had been made including 517 in vaccinated and 202 in unvaccinated children. Ongoing surveillance through the HRU, CDSC, Scottish Centre for Infection and Environmental Health (SCIEH) and OVG to the end of March 2001 identified 67 additional cases (52 vaccinated, 15 unvaccinated). Of this total, 225 cases represented true vaccine failures (TVFs). Two hundred and sixteen of the 225 TVF were vaccinated in the first year of life: 202 received three doses and 14 received two doses. Nine were vaccinated when older than 12 months of age. Medical and immunological conditions amongst the cases of TVF are detailed in Table 3.2. Of the 24 children with clinical risk factors, 8 had underlying malignancy, 5 a dysmorphic syndrome, 3 Down's syndrome, 2 a chromosomal anomaly and 1 Neutropaenia. Five children had other specific conditions identified as increasing their risk of infection.

Table 3.2: Associated conditions of TVF Sept 1992-March 2001

Associated condition	Number of cases where information known	Number with risk factor (% of known)
Prematurity	206	22 (10.7%)
Immunoglobulin Def'cy	137	36 (26.3%)
Clinical Risk Factor (all)	203	24 (11.8%)

Convalescent sera were available in 163 cases of TVF. Forty-three (26%) demonstrated a convalescent antibody response less than 1.0 μg/ml, the level of antibody thought to correlate with long-term clinical protection. Of these, information on responses to a booster dose of vaccine was available in 24 cases, of whom all but one achieved an antibody level above this 'protective' threshold (McVernon J et al 2001).

3.2.2 Immunologic memory and Hib vaccine failure

Methods

This study aimed to compare the geometric mean concentration of convalescent PRP-specific serum antibodies produced following invasive Hib infection between Hib conjugate vaccine immunised and unimmunised children. In making this comparison, significant confounding factors influencing the immune response to disease were taken into account.

Subject Populations

A. UK Hib vaccine failures

Subjects were identified through enhanced surveillance for invasive Hi infections using methods and case definitions described earlier. All children included in this analysis had confirmed invasive Hib infection following receipt of three doses of Hib vaccine in infancy, at 2, 3 and 4 months of age, without a booster dose. Additional clinical information was prospectively collected on the cases as part of the BPSU study. Serum was collected for measurement of acute and convalescent concentrations of Hib antibody and the timing of collection in relation to disease onset was noted. Measurement of immunoglobulin classes and subclasses was also performed on convalescent specimens and classified as normal or deficient in relation to age appropriate reference ranges. This study was approved by the Central Oxford Research

Ethics Committee in 1991 and again by the South East Multi-Centre Research Ethics Committee in 2001.

B. Unimmunised Australian children with Hib

A cohort of children, previously described (Johnson PDR et al 1996), was selected as an unvaccinated historical control group from among patients admitted to the Royal Children's Hospital, Melbourne, Australia between February 1988 and August 1990 with Hib epiglottitis or meningitis. Of 47 children with meningitis, 46 had positive Hib cultures from blood or CSF or both. Forty-five cases of epiglottitis were described – 32 had a positive blood culture, 8 a positive throat swab and a further 3 were diagnosed on latex antigen testing. Two were diagnosed on clinical grounds alone according to the judgement of two experienced clinicians. The GMC of convalescent Hib antibodies following epiglottitis in the previous study was no different when children without a positive blood culture were included in the case definition, compared with the GMC for the 32 from whom Hib was grown in the blood. No children had received Hib vaccine. Acute and convalescent serum samples were taken and age at presentation, clinical diagnosis and timing of serum collection were also noted. The study was approved by the Ethics in Human Research Committee of the Royal Children's Hospital, Melbourne.

Antibody Measurement

In the UK, PRP antibody measurements were performed initially using the Farr type radioimmunoassay (Booy R et al 1992) with a change to the Centres for Disease Control (CDC) standard protocol HbO-HA ELISA (Phipps DC et al 1990) in 1999. Equivalency studies performed at the time were favourable, with some discordance noted in the lowest ranges only (H Griffiths, personal communication). All assays in Australia were conducted using the HbO-HA ELISA. Both assays were standardised

using reference sera supplied by the Food and Drug Administration, US (Phipps DC et al 1990) (Booy R et al 1992).

Statistical Analysis

For descriptive statistics, the Wilcoxon ranksum test was used to compare group medians. In the comparison of Hib antibodies, a minimum concentration of 0.08 μg/ml was assigned for values <0.15 μg/ml. Convalescent antibody measurements were log transformed in order to achieve a normal distribution and described as GMCs. For the pooled data on vaccinated and unvaccinated children, simple regression analysis was performed to assess the relationships between convalescent antibody and both age at presentation and timing of serum collection. These were then incorporated into a multiple linear regression model, which was used to predict adjusted convalescent antibody responses. Student's t test was used to assess the significance of any difference in raw and adjusted GMCs between vaccinated and unvaccinated children. The same comparisons were made with children presenting with either meningitis or epiglottitis considered separately.

Further exploration of potential confounders was performed using the UK vaccinated dataset. The effects of the various clinical and immunologic variables measured on logged convalescent antibody concentrations were first assessed using simple linear or logistic regression analysis depending on the nature of variables assessed. Their relative contributions were then further measured in a multivariate regression model in which all potential confounders were included. All statistical analyses were performed using STATA 7.0 (STATA 2001).

Results

From October 1992 to 1st January 2001, 185 reports of Hib vaccine failure following three doses in infancy were notified through surveillance in the UK and Republic of Ireland (171 in the UK). Forty-nine of these presented with illnesses other than epiglottitis or meningitis, such as pneumonia, bacteraemia, cellulitis or bone and joint infections, and were excluded. Children born prematurely or on whom gestational information was unavailable were considered separately (3 of 38 with epiglottitis, and 13 of 98 with meningitis). Children with incomplete antibody data, for either concentration or day of collection (a further 27), were also excluded. This left 25 vaccinated children presenting with epiglottitis and 68 with meningitis. One hundred and twelve cases were described in the study of unvaccinated children. Clinical presentation was unknown for one, and no convalescent serum was available for 19, leaving 45 children with epiglottitis and 47 with meningitis.

Demographic characteristics of the two groups are shown in Table 3.3. Vaccinated children presenting with meningitis were 8.5 months older than their unimmunised counterparts ($p=0.005$) and had earlier blood sampling at 28 days, compared with 52 days ($p<0.001$). It should be noted, however, that a wide range of ages and sampling times was observed in both groups. Raw antibody data for all the children, plotted against age at presentation with disease, are shown in Figure 3.2. There was a statistically significantly higher concentration of antibodies in the vaccinated group ($p<0.001$) (Table 3.4). This was most marked in infants under 18 months of age. Only 13% of unvaccinated children in this age group made any antibody response to infection, compared with 92% of those who had been immunised. However, age at presentation and timing of serum collection were both significantly related to antibody

concentrations in the multiple regression model ($p<0.0001$ for both) (Table 3.5). Increasing age was associated with an exponential increase in antibody levels, and antibodies were seen to decay exponentially with time from illness. When antibody levels were adjusted for these confounding factors, a significant difference between the convalescent antibody responses of immunised and unimmunised children was only observed in those presenting with meningitis ($p=0.0003$) (Table 3.4).

Relationships between the immune response to Hib meningitis or epiglottitis and the additional clinical risk factors of chronic illness, known immunodeficiency, premature delivery and deficient immunoglobulins or subclasses described in UK vaccine failures were explored (data not shown). The 93 vaccinated children studied above, as well as 11 prematurely born children with complete data were included. The only additional factor found to influence the magnitude of the convalescent response was a history of premature delivery (coefficient –2.57, coefficient standard error 0.74, $p=0.0008$), which remained significantly associated with lower antibody levels in the multiple regression model ($p=0.047$). Comparison of raw Hib GMCs confirmed this finding. Children born prematurely had a GMC of 0.82 μg/ml (95% CI 0.27, 2.54), while in those born at term it was 10.81 μg/ml, (6.62, 17.66); ($p=0.008$). This difference persisted after adjusting for age and timing of serum collection: preterm GMC 4.17 μg/ml (2.39, 7.28); term GMC 8.93 μg/ml (7.00, 11.38); $p=0.04$, although overlapping confidence intervals are noted. For this reason, children delivered prematurely were considered a population of outliers and excluded from the earlier comparisons with the unvaccinated cohort. Their inclusion in the analysis resulted in lower GMC Hib antibody responses in the vaccinated group than those described in Table 3.4. It did not, however, change any of the significant differences observed between groups or conclusions drawn.

Table 3.3: Demographic characteristics of vaccinated and unvaccinated children

Vaccination status	Clinical presentation	Number of cases	% Female	Median day of convalescent serum collection (range)	Median age in months (range)	% less than 18 months
Vaccinated	Epiglottitis	25	56%	41 (9, 300)	36.89 (11.93, 63.12)	12%
	Meningitis	68	46%	28 (8, 135)	25.23 (7.27, 91.30)	31%
Unvaccinated	Epiglottitis	45	44%	43 (27, 78)	31.79 (7.50, 98.20)	11%
	Meningitis	47	46%	52 (16, 112)	16.87 (1.41, 71.64)	53%

Figure 3.2: Log of Raw Convalescent Hib Antibody Concentrations (mcg/ml) for Vaccinated and Unvaccinated Children

Individual data points for vaccinated children are represented by x, with the dashed line indicating line of best fit for this group. Individual data points for unvaccinated children are represented by o, with the dotted line indicating line of best fit for this group.

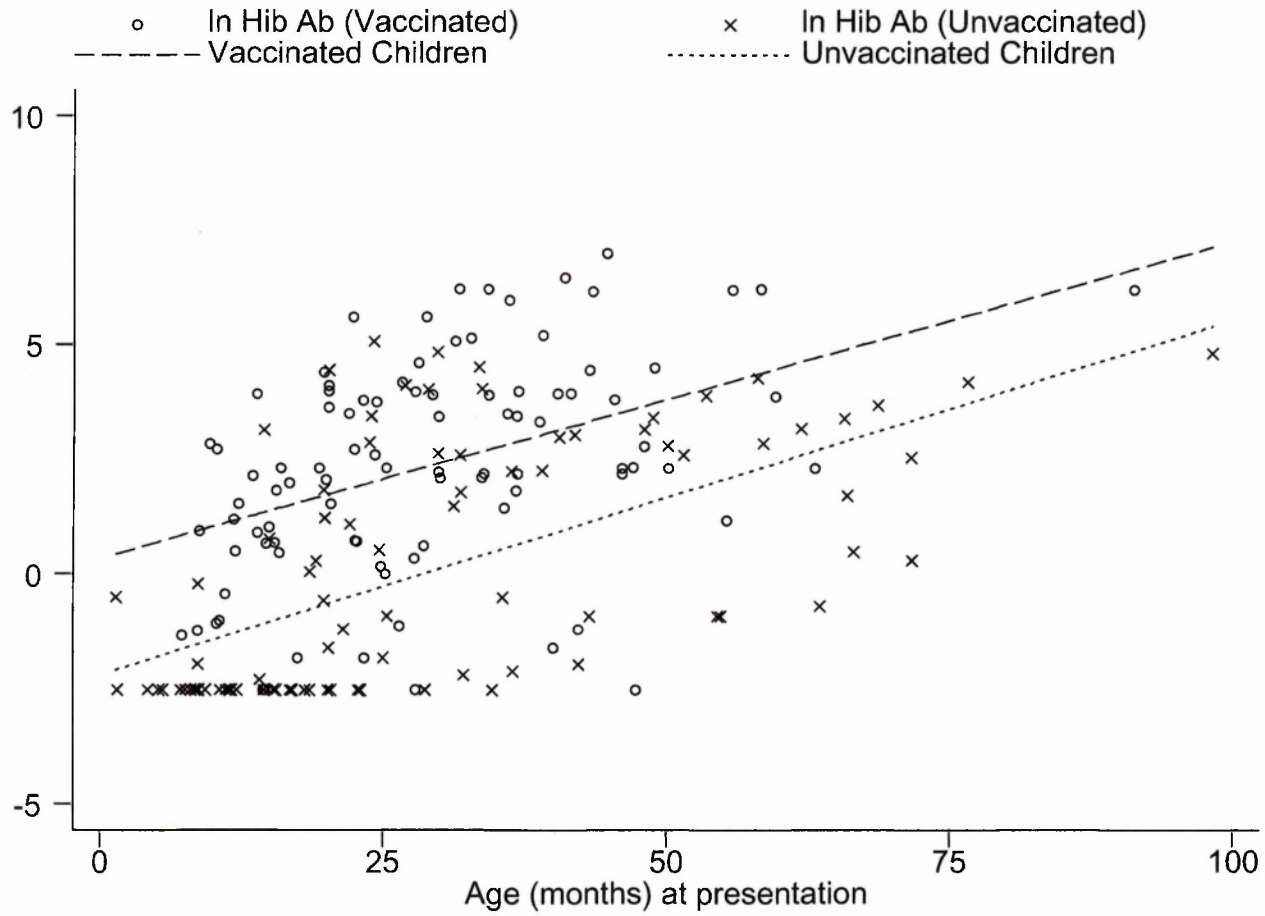


Table 3.4: Convalescent Hib Antibody Geometric Mean Concentrations, with 95% Confidence Intervals

Vaccination status	All Cases		Meningitis Cases Only		Epiglottitis Cases Only	
	Raw	*Adjusted*	*Raw*	*Adjusted*	*Raw*	*Adjusted*
Vaccinated	10.81 (6.62, 17.66)	3.86 (2.96, 5.03)	8.32 (4.47, 15.46)	3.78 (2.78, 5.15)	22.08 (11.18, 43.61)	4.06 (2.32, 7.12)
(no. cases)	93	93	68	68	25	25
Unvaccinated	1.06 (0.61, 1.84)	3.01 (2.14, 4.23)	0.33 (0.18, 0.60)	1.48 (0.90, 2.21)	3.57 (1.60, 7.99)	6.32 (3.92, 10.20)
(no. cases)	92	92	47	47	45	45
Student's t test p value*	<0.0001	0.25	<0.0001	0.0003	0.003	0.25

Table 3.5: Effects of Age at Disease Presentation and Timing of Convalescent Serum Collection on Convalescent Hib Antibody Response (Vaccinated and Unvaccinated Children)

a. Simple regression

Variable	Intercept	Coefficient	Coefficient SE	p Value	Adjusted r^2
Age (months)	-0.97	0.08	0.01	<0.0001	22.6%
Convalescent day of serum collection	2.15	-0.02	0.01	0.002	4.4%

b. Multiple regression – Adjusted r^2 = 27.8%, p<0.0001

Variable	Coefficient	Coefficient SE	p Value
Age (months)	0.08	0.01	<0.0001
Convalescent day of serum collection	-0.02	0.01	<0.0001

3.2.3 National case-control study of social and environmental factors associated with all invasive Hib infections of childhood, 1998-2002

Methods

A national postal questionnaire study was conducted in order to look for risk factors associated with ongoing invasive Hib infections in the UK over the period 1998-2002, using a matched case-control design. This study was conducted within the Immunisation Department of the HPA's Communicable Disease Surveillance Centre (CDSC).

Case definition

A sample of cases from the combined CDSC/HRU dataset of confirmed invasive Hib infection was defined for this study. Children presenting in England between 1st January 1998 and 1st January 2003 who had survived their disease were selected, regardless of vaccination status. In order to ensure equivalent access to immunisation, only those born after 1st January 1993 were eligible for inclusion.

Control identification

Child health system managers and District Immunisation Co-ordinators were advised of the study by email in December 2002. Letters were subsequently sent to child health contacts in regions where a case had been identified, asking them to nominate 5 children on their computer system who were born on the same day as the index case. Immunisation dates and batch numbers were requested, along with the name of the child's registered General Practitioner. The Health Protection Agency has approval under Section 60 of the Health and Social Care Act to process such confidential patient information for the purpose of monitoring the efficacy and safety of vaccination programmes.

Approach to Subjects

A two-stage consent process was requested by the ethics committee. In the first instance, a letter was sent to the usual General Practitioner of all potential study subjects, asking consent to release of the name and address of the child's parents, and enquiring after the appropriateness of an approach to the family at the present time. Practices were also asked to check vaccine dates and batch numbers, as supplied by child health contacts. Following receipt of written informed consent from the GP, an invitation letter and questionnaire were sent directly to the parents of each child.

Study Procedure

A questionnaire inquiring after a range of clinical and environmental risk factors was sent to the parents of each study subject. Information requested included:

- Perinatal data: date of birth, birthweight, sex, gestation, breast feeding history
- Medical history: history and nature of chronic illness, antibiotic use over the first years of life (never, once/twice yearly, every few months, almost every month), vaccination dates and batch numbers
- Child exposure: type of day care (home only, childminder or nursery, other) and number of other children in that care from ages 0-2 years, 2-5 years (<5, 5-10, >10)
- Household: parents at home, siblings, bedroom sharing, smokers, presence or absence of central heating, home ownership (own outright, own with mortgage, rents, lives rentfree)
- Parental information: country of birth, ethnicity, occupational status

Parents were asked to return the completed questionnaire along with their written informed consent using a reply paid slip. They were encouraged to seek further

information from the study investigators by telephone, or to speak with their own general practitioner or health visitor if they had any questions regarding the study.

Parental occupations were coded according to the Standard Occupation Classification 2000 (SOC2000) published by the Office for National Statistics (ONS) (http://www.statistics.gov.uk/nsbase/methods_quality/ns_sec/soc2000.asp). Each parent was then assigned a National Statistics Social and Economic Classification (NS-SEC) from the reduced derivation tables provided by the ONS, on the basis of the SOC2000 and their employment status. Operational categories of the NS-SEC were linked to Social Class, according to tables available on the ONS website (http://www.statistics.gov.uk/methods_quality/ns_sec/contents.asp). In keeping with recent changes in the allocation of household level SEC, the parent with higher earning potential regardless of gender was assigned to be the household reference person, by whose status the family's social class was determined.

Vaccines were identified from batch numbers, using information supplied by the manufacturers as described earlier in Section 3.1.2. If the manufacturer's product could not be identified with certainty from the batch number provided, no code was assigned. As in the earlier analysis of batch numbers, the primary question of interest was whether DTaP-Hib was over-represented among cases compared with controls. The point of repeating the study within this design was to allow matching by area as well as date of birth in order to account for possible regional variation in vaccine distribution. In keeping with the period of use of DTaP-Hib in the UK, only cases born after 1st October 1999 who had received three identifiable doses of a Hib vaccine before disease onset were included in the analysis.

Statistical methods and sample size

Questionnaire data were entered into a relational Dataease database maintained at CDSC on a secure network. The month and year of birth were used as the basis for matching cases with controls. Univariable conditional logistic regression models were used to examine the relative contribution of the exposures of interest to the risk of disease. These relationships were described as odds ratios (ORs) with 95% confidence intervals (CIs). Variables significant in the first analysis at the level of p=0.05 or less were then included in multivariable models. Each additional exposure was later incorporated singly to see whether any achieved significance within this context. Where two exposures were strongly correlated, only the most significant was used in the analysis. Relationships with factors described in terms of a number of levels, such as antibiotic use, were explored using all tiers, and as tests for trend.

For the study of vaccine batches, conditional logistic regression was used to compare the number of doses of the full primary immunisation series given as DTaP-Hib between each case and their birth-date and regionally matched controls. All statistical analyses were conducted using STATA 7.0 (STATA 2001).

An unmatched study with a sample size of 332 cases and 664 controls would allow identification of an OR of 1.5 for an exposure with a control prevalence of 30% with 80% power and 95% confidence. Such an OR has been previously quoted for the additional risk of invasive disease conferred by a smoking household member (Cochi SL et al 1986), the prevalence of which is 30% in Oxfordshire (Dr M Barbour, personal communication). Odds ratios for other risk factors identified have been much higher, for instance, 4.0 for day care attendance, 2.5 for household crowding and 0.3 reflecting

reduction of risk attributable to breast feeding (Cochi SL et al 1986). Given the reported prevalence of each of these exposures in a British population, only 40-60 cases (and twice as many controls) would be needed to find similar risks with 90% power and 95% confidence. The use of a matched design further increased the study's power.

Results

Three hundred and thirty-two cases were identified which met the selection criteria. Names of potential controls were released by child health contacts as requested in relation to 301. Of the remaining 31, controls for 18 cases were approached directly by the PCT in line with individual trust guidelines. No controls could be identified for the remaining 13. In all, names of 1,350 potential controls were obtained.

Twenty-three of the cases were not currently registered with a GP in England according to child health records. Of the 309 remaining, up to four letters were sent to GPs requesting consent to parental approach. By the end of December 2003, responses were received in relation to 264 children (85%), with only 14 refusing consent to approach parents. Letters were addressed to the doctors of 1,350 potential controls. 1,060 (79%) replied, with 88 refusals and 972 consents. Parents could be sent a maximum of two letters in line with ethical guidelines on approach of subjects. One hundred and thirty of 250 cases (52%) replied to the invitation, with 127 (51%) agreeing to participate. Nine hundred and seventy-two controls were approached – 276 (28%) replied and 259 (27%) took part.

The distribution of variables in the population and ORs derived from the univariable analyses, matched by month of birth, are shown in Table 3.6. Exposures initially included in the multivariable model were: prematurity, antibiotic use, number attending

nursery between 2-5 years, bedroom sharing, number of parents at home, central heating and vaccination status. Number of children in nursery from 0-2 years was not included, as a significant correlation was found between nursery attendance at different ages. Bedroom sharing and central heating were dropped from the model as they did not make a significant contribution to risk. The number of smokers in the household was added as it was found to be significantly associated with outcome. Results of the final multiple regression model are shown in Table 3.7.

A history of prematurity and living in a single parent household were the strongest risk factors for invasive Hib infection. Frequent antibiotic use was also associated with increased likelihood of disease. Attendance at a day care nursery between the ages of 2 and 5 years appeared to reduce the risk of infection, decreasing by 20% with each additional increment in the number of children cared for in the same environment. Surprisingly, having smokers in the household was also associated with a reduced risk of invasive infection. Vaccination provided the best protection in this analysis.

Findings of the study of vaccine batches received by cases and controls, matched by exact date of birth and region, are shown in Table 3.8. Due to the stringency of matching and restriction of dates of birth for the purpose of this analysis, only 49 observations were included, resulting in substantial loss of power. In spite of this loss of data, a clear trend to increasing risk of vaccine failure with each additional dose of DTaP-Hib vaccine received in the infant primary course was observed. A doubling of risk occurred for each increment, with only a 6% probability that this finding was due to chance.

Table 3.6: Distribution of variables, results of univariable analyses

Variable	Case	Control	Odds Ratio (95% CI)	p value
Perinatal Data				
Birthweight (g) mean (95% CI)	3413 (3296, 3530)	3435 (3356, 3514)	Student's t test	p=0.75
Sex	F=56, M=75	F=119, M=147	0.97 (0.61, 1.54)	p=0.90
Prematurity	No=120, Yes=11	No=253, Yes=13	3.37 (1.12, 10.1)	p=0.03
Breastfeeding (BF)	No=45, Yes=86	No=88, Yes=178	0.93 (0.56, 1.54)	p=0.78
Median months BF (range)	6 (1, 30)	4.5 (1, 36)	Ranksum test	p=0.23
Medical History				
History of illness	No=99, Yes=32	No=217, Yes=48	1.26 (0.71, 2.21)	p=0.43
Antibiotic Use (stratified)	Never=27 1-2/year=71 Few mos=19 Monthly=10	Never=73 1-2/year=160 Few mos=24 Monthly=7	1.0 1.32 (0.73, 2.38) 2.35 (1.0, 5.51) 3.65 (1.18, 11.3)	p=0.06
Antibiotic use (trend)			1.54 (1.12, 2.12)	p=0.008
Vaccination†	No=10; Yes=121	No=1; Yes=265	0.03 (0.005, 0.27)	p=0.001
Child Exposure				
Number of children in care 0-2 yrs (stratified)	0=95 <5=13 5-10=2 >10=21	0=170 <5=24 5-10=7 >10=64	1.0 1.05 (0.46, 2.4) 0.27 (0.03, 2.43) 0.62 (0.34, 1.14)	p=0.25
Number of children in care 0-2 years (trend)			0.84 (0.69, 1.03)	p=0.09
Number of children in care 2-5 yrs (stratified)	0=52 <5=6 5-10=5 >10=56	0=67 <5=10 5-10=14 >10=153	1.0 0.57 (0.16, 2.02) 0.38 (0.12, 1.22) 0.41 (0.23, 0.74)	p=0.02
Number of children in care 2-5 yrs (trend)			0.75 (0.62, 0.90)	p=0.002
Household				
Parents home	Both=106 Sole parent=25	Both=242 Sole parent=24	2.11 (1.03, 4.33)	p=0.04
Number of siblings (stratified)	0=22 1=68 2=31 3=7 4 or more=3	0=69 1=133 2=46 3=16 4 or more=2	1.0 1.74 (0.93, 3.3) 1.79 (0.87, 3.68) 1.06 (0.35, 3.2) 5.23 (0.48, 56.8)	p=0.26
Number of siblings (trend)			1.17 (0.90, 1.52)	p=0.24
Bedroom sharing (stratified)	0=77 1=43 2=6 3 or more=5	0=195 1=60 2=9 3 or more=2	1.0 1.49 (0.88, 2.53) 1.60 (0.53, 4.82) 4.75 (0.83, 27.2)	p=0.14
Bedroom sharing (trend)			1.47 (1.05, 2.05)	p=0.03
Smokers (stratified)	0=96 1=22 2=11	0=190 1=51 2=20	1.0 0.66 (0.36, 1.2) 0.87 (0.36, 2.1)	p=0.38
Smokers (trend)			0.83 (0.57, 1.2)	p=0.32
Heating	No=11, Yes=120	No=14, Yes=252	0.54 (0.21, 1.4)	p=0.19
Own or Rent (stratified)	Own outright=11 Mortgage=86 Rents=30 Rentfree=3	Own outright=19 Mortgage=207 Rents=38 Rentfree=2	1.0 0.60 (0.25, 1.44) 1.06 (0.39, 2.9) 1.8 (0.24, 13.6)	p=0.16
Own or Rent (trend)			1.3 (0.85, 2.01)	p=0.23

Variable	Case	Control	Odds Ratio (95% CI)	p value
Parental Variables				
Maternal Ethnicity (White British/Irish vs Other)	Mother White: 125 Mother Other: 6	Mother White: 248 Mother Other: 18	0.76 (0.28, 2.04)	p=0.58
Paternal Ethnicity (White British/Irish vs Other)	Father White: 122 Father Other: 6	Father White: 250 Father Other: 14	1.04 (0.33, 3.22)	p=0.95
Household Social Class (stratified)	I=13 II=50 III=48 IV=10 V=2	I=23 II=137 III=82 IV=12 V=2	1.0 0.45 (0.19, 1.06) 0.70 (0.29, 1.7) 1.23 (0.34, 4.4) 1.21 (0.12, 12.5)	p=0. 12
Household Social Class (trend)			1.20 (0.88, 1.65)	p=0.25

Table 3.7: Results of multivariable analysis

Variable	Odds Ratio (95% CI)	p value
Prematurity	4.45 (1.3, 15.6)	p=0.02
Single parent household	2.61 (1.1, 6.3)	p=0.03
Antibiotic use (trend)	1.59 (1.1, 2.4)	p=0.02
Number of children in nursery age 2-5 yrs (trend)	0.78 (0.64, 0.96)	p=0.02
Number of smokers in the Household (trend)	0.55 (0.33, 0.91)	p=0.02
Vaccinated	0.02 (0.002, 0.29)	p=0.003

Table 3.8: Analysis of vaccines received, controls matched by DOB and region

Variable	Case	Control	Odds Ratio (95% CI)	p value
Number of Doses of aP Combination Vaccine (stratified)	0=14 1=3 2=5 3=22	0=19 1=9 2=12 3=36	1.0 0.96 (0.05, 20.3) 5.3 (0.18, 153) 6.6 (0.3, 133)	p=0.25
Number of Doses of aP Combination Vaccine (trend)			2.1 (0.9, 5.0)	p=0.06

Discussion – host and environmental factors

Studies of risk factors associated with invasive Hib infection have sought to define populations of susceptible children who may be a source of ongoing transmission of the organism, or be at heightened individual risk of developing invasive disease if exposed. Identification of such groups may direct targeted interventions in order to enhance the impact of the routine Hib immunisation schedule.

Examination of clinical factors associated with ongoing Hib disease in the vaccine era in the BPSU/OVG study revealed that the majority of children presenting with Hib vaccine failure appeared perfectly healthy before they developed their infection. Only 1 in 10 had a pre-existing medical diagnosis clearly associated with reduced immune function that would have prompted their usual clinicians to consider them at heightened risk. As such, an intervention strategy aimed at boosting the immunity of children with these diagnoses may have been individually beneficial, but would not have prevented the bulk of vaccine failure cases.

A further finding of the BPSU/OVG study was the observation that approximately 1 in 4 recovered Hib vaccine failures showed some minor deficiency of immunoglobulin classes and subclasses. This replicated similar reports from studies conducted elsewhere (Holmes SJ et al 1991) (Breukels MA et al 2001). Immunoglobulin subclass deficiency was interpreted as potentially significant in light of an earlier established but inconsistent link with hyporesponsiveness to polysaccharide antigens and recurrent sinopulmonary infection (Shackelford PG et al 1990). The most obvious limitation of a finding established only by serum testing in convalescence was the inability to intervene prospectively to prevent such cases. The other difficulty of interpretation was

establishing whether children with subclass deficiencies, or indeed any of those presenting with conjugate vaccine failure, showed any quantitative or qualitative abnormality in their response to immunisation.

Post primary immunisation (5 months) antibody titres were not available for children later presenting with invasive Hib infection, as these were not routinely taken or stored. Antibody titres collected during the acute phase of disease were not an accurate reflection of immunity prior to exposure to the organism, and were very difficult to interpret. Reasons for this difficulty included an uncertain duration of Hib colonisation prior to invasive disease, which may have produced some natural immunity. Many unimmunised children over the age of 2 years presenting with Hib meningitis in the 1970s had substantial specific antibody elevations within only a few days of disease onset (Anderson P et al 2000). Coexistence of bactericidal activity in serum and bacteraemia (Schneerson R et al 1971) was postulated to be due to constant seeding of the organism from extravascular tissues, in excess of capacity for clearance (Shaw S et al 1976). Conversely, binding of antibody by persisting free PRP antigen would be expected to reduce measurable titres. Circulating free PRP has been regularly observed for between 1 and 30 days of convalescence, and may be found for many months in some individuals (O'Reilly RJ et al 1975).

Some measure of priming by infant immunisation was sought by comparing the convalescent antibody response to Hib disease as a 'booster' in previously vaccinated British children with an unvaccinated Australian historical cohort. After correction for the confounding effects of age and timing of serum collection (Johnson PDR et al 1996), a significant difference in the magnitude of the immune response between

vaccinated and unvaccinated children was only seen in those presenting with Hib meningitis (McVernon J et al 2003b). We attributed this difference to priming for immunologic memory by infant immunisation, without clinical protection from disease.

Prior to the use of conjugate vaccines, it was noted that children with epiglottitis tended to be older and have higher convalescent anticapsular antibody titres than those with meningitis (Whisnant JK et al 1976). It has been suggested that the presence of pre-existing immunity in these cases was enough to contain invasive disease at the epiglottis, perhaps as a result of immunologic priming through prior oropharyngeal Hib carriage (Johnson PDR et al 1996). The absence of a difference in convalescent GMCs between vaccinated and unvaccinated children with epiglottitis may support the notion that both groups were primed. In the Australians, this priming was most likely the result of carriage of Hib or cross reactive organisms, and in the UK cohort, may have been attributable to vaccination with or without carriage. Alternatively, the greater immune response to infection in this group may simply reflect immunologic maturation of the response to capsular polysaccharides with age.

Ten of 25 children in a previous study of Hib vaccine failures had low levels of IgG2 subclass and IgM (Holmes SJ et al 1992), and also showed a reduced convalescent antibody response to disease. In the present series, no such difference in Hib antibody concentrations was noted in children with abnormalities of immunoglobulins or subclasses, using multiple regression analysis. However, the possibility that other defects in immune regulation may be over-represented among vaccine failures deserves further exploration. A range of polymorphisms in genes responsible for regulating antibody responses, initiating or modulating pathogen recognition or clearance have

been implicated in conferring such susceptibility to a range of diseases (Kwiatkowski D 2000).

A history of premature delivery had a marked effect on antibody response in this study. Some of the reduction in titres may be explained by the lower age of this subset with a median age of 20 months (range 10-51) compared with those delivered at term [28 months, (range 7-91)]. However, persistently lower antibody levels were observed, even following adjustment for age and timing of serum collection. These results would strongly suggest defective induction of memory in our population of premature infants. Lower primary antibody responses (Slack MH et al 2001) and reduced persistence (Heath PT et al 2003) have been observed previously following vaccination of premature infants with conjugate vaccines. These new data added further weight to the suggestion that prematurity was a risk factor for vaccine failure (Heath PT et al 2003).

The national case control study of social and environmental factors enrolled fewer subjects than initially hoped. Significant barriers to recruitment arose as a result of ethical constraints imposed, requiring multiple consent procedures at primary care trust, practitioner and patient stages. In spite of the associated loss of power, a number of variables were found to be significantly associated with altered risk of invasive Hib infections in the UK in an era of widespread vaccination coverage.

Within this study design, a history of prematurity was significantly linked with an increased likelihood of disease [OR 4.45 (1.3, 15.6) p=0.02], in keeping with the evidence of reduced vaccine immunogenicity described above. Living in a sole parent household also conferred additional risk [OR 2.61 (1.1, 6.3) p=0.03]. The same finding

had been noted from the US [OR 4.3 (1.2, 14.8)] (Jafari HS et al 1999) and Switzerland [OR 3.52 (1.0, 15.37] (Muhlemann K et al 1996) in communities with partial vaccine coverage. The way in which this effect is mediated in divergent populations may differ, and the mechanism was unable to be defined in this study. Incomplete immunisation was more common in children residing in single parent households in the US thereby clearly increasing disease risk (Jafari HS et al 1999), although no such relationship was seen in the UK. Antibiotics were more frequently prescribed over the first years of life for children subsequently presenting as Hib cases [OR (trend) 1.59 (1.1, 2.4)]. This finding may reflect the fact that children prone to infections may be expected to attend medical practitioners more often with illnesses requiring antibiotic therapy.

An unanticipated protective effect of nursery attendance in children aged between 2 and 5 years was found, rising by 20% with each incremental increase in the number of children in care (OR (trend) 0.78 (0.64, 0.96) p=0.02]. This was in marked contrast to reports from the US in the 1980s of heightened risk of both primary and secondary Hib cases in such settings, most marked in children under 2 years of age. Different estimates were obtained between geographical areas in these studies, which were attributed to variation in the size of centres, and definitions of classroom contact (Broome CV et al 1987b). While no studies of community-based risk factors for Hib disease were conducted in the UK prior to vaccine introduction, it is unlikely that characteristics of day care on both sides of the Atlantic would be so different as to have opposite effects on outcome. Of note, a Swiss study involving predominantly unvaccinated children around the time of vaccine introduction showed a similar trend to reduction of risk of Hib infection in children over the age of 2 years attending nurseries or schools (Muhlemann K et al 1996). The authors of this study attributed their finding

to increased opportunity for exposure to cross reactive antigens in this situation. Alternatively, it may be proposed that children able to mount an antibody response to Hib, either through maturation of natural immunity to polysaccharides or priming by conjugate vaccines, may benefit from repeated boosting of specific antibody associated with episodes of Hib colonisation.

The further unexpected finding of a protective effect of having smokers in the household [OR (trend) 0.55 (0.33, 0.91) p=0.02] may be postulated to act through a similar mechanism. Passive smoking has been linked with carriage of multiple strains of nt Hi (StSauver J et al 2000). If children of smokers are more heavily colonised with respiratory pathogens, this increased exposure may boost natural immunity. Alternatively, some unidentified confounding factor may be responsible for the observed association.

Vaccination was strongly protective against infection in the case-control study, the odds ratio of 0.04 corresponding with 96% direct efficacy. This figure should be interpreted with caution, as vaccine coverage in the control population recruited was 99%. Such a high level of immunisation uptake was well above the 91-93% observed in the UK over the past 10 years, indicating that our sample was unfortunately not truly representative of the population (Figure 2.1). On this basis, the estimate of efficacy cannot be directly compared with figures derived in other studies (Ramsay ME et al 2003).

3.3 Changes in population immunity in the UK 1990-2002, possible consequences for transmission and invasive disease

In considering possible aetiologic causes of the change in Hib epidemiology over time, it is worth reiterating the vaccines's impact on transmission of the organism, with a subsequent reduction in boosting of natural immunity. Figures 3.3a and 3.3b summarise the shifts in prevalence of specific antibody to Hib in the population in the past ten years, as measured in the HPA studies (Section 2.6.2).

Figure 3.3a: Seroprevalence of Hib antibody titres ≥1.0 μg/ml by age, 1990/1994

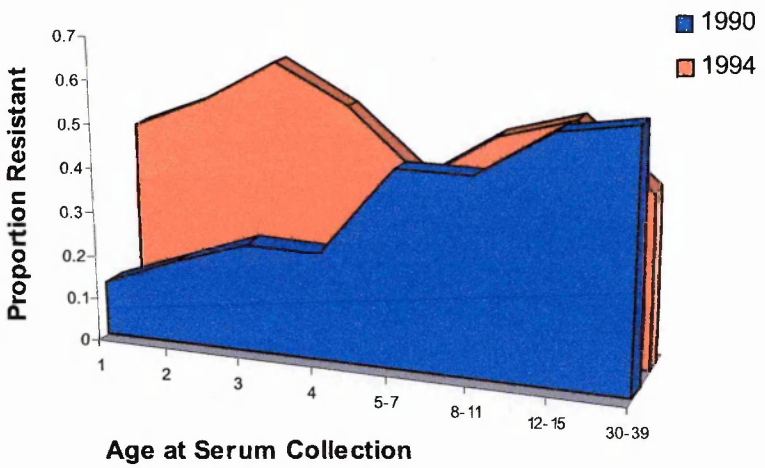
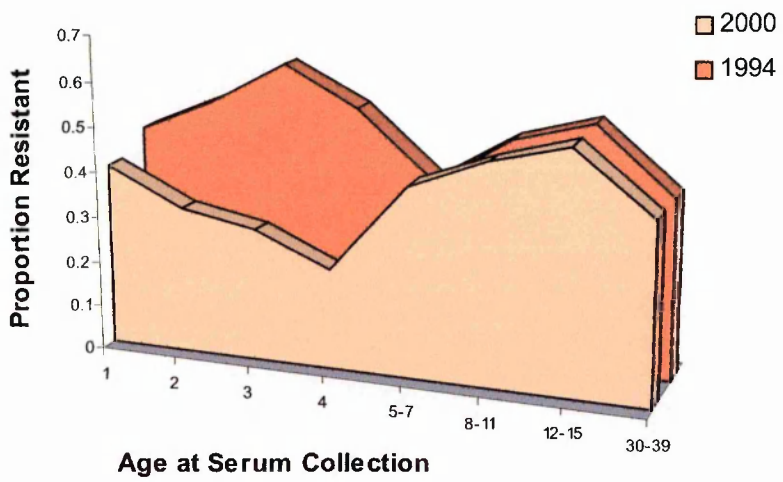


Figure 3.3b: Seroprevalence of Hib antibody titres ≥1.0 μg/ml by age, 1994/2000



The reduction of antibody concentrations in toddlers among cohorts immunised in infancy (Figure 3.3b) has been seen to result in lower direct protective efficacy of vaccine (Section 3.1.1). Effects on herd immunity resulting from this change remain incompletely understood. In addition, the potential for susceptibility to re-emerge in older age groups (Figure 3.3a) may contribute to a further pool of potential transmitters of the organism at the opposite end of the age spectrum. The cost of population based carriage studies to monitor such changes on an ongoing basis is prohibitive, particularly when carriage prevalence is low. Further, even with persistently low levels of colonisation in the population, lower immunity at the point of exposure to the organism may lead to a proportional increase in cases arising from a given number of carriage episodes. Mathematical models of the impact of immunisation on Hib transmission and immunity have been developed in order to explore these issues, and will be outlined in more detail in subsequent chapters.

3.4 Conclusion

Vaccine-related, host and environmental factors potentially contributing to ongoing Hib infections in the UK and their changing epidemiology over time have been explored in a series of studies conducted by the HPA, OVG and BPSU.

Direct efficacy of the accelerated infant immunisation schedule employed in the UK has been lower than anticipated from phase II trials, at 61% in the first two years following vaccination, waning to 27% thereafter. This deficiency was masked for several years by the use of catch-up immunisation in the UK in 1992 to 1993, with an estimated short-term protective effect of 97%. Superimposed on waning of the catch-up phenomenon

was the use of a less immunogenic DTaP-Hib vaccine that was both temporally associated with a reduction in efficacy in the population, and correlated with an increase in the odds of vaccine failure in a matched case-control analysis. Each additional dose of DTaP-Hib given as part of the full infant primary schedule conferred a 2-fold increased risk of vaccine failure in two separate study designs. These changes in measured immunogenicity and efficacy corresponded closely with the population experience of paediatric invasive Hib disease.

Cohort and case-control studies were conducted in order to understand more of the mechanism of ongoing Hib disease and vaccine failure, and to potentially identify subpopulations that may benefit from additional measures to prevent Hib infection. The majority of children presenting with Hib in the vaccine era appeared healthy before their disease, with only 1 in 10 reporting a preceding clinical diagnosis associated with increased risk of bacterial infections in the cohort study. A significant association was found between frequent antibiotic use and Hib disease in the case-control study, suggesting that some degree of enhanced susceptibility to infection may have been present in a subgroup, in the absence of a definable clinical syndrome. In keeping with this hypothesis, immunoglobulin subclass deficiency was found in the convalescent serum of 25% of children presenting with Hib despite vaccination. This immunophenotype was of uncertain prognostic signficance, however, and was not associated with evidence of impaired priming by infant immunisation, as evidenced by the magnitude of the convalescent antibody response to invasive infection. Overall, Hib vaccine failures appeared to have been successfully primed for memory immune responses by infant immunisation, but were still not protected from disease.

Measures of Hib vaccine immunogenicity following the infant primary course are reduced in children delivered prior to term. Evidence of deficient priming in infancy was further suggested by a marked reduction in convalescent Hib titres among vaccine failures with a history of prematurity, compared with those delivered at term. A previous analysis of the relationship between prematurity and vaccine failure in the OVG cohort study failed to show a significant contribution of this risk factor to disease. In the national case-control study, however, premature delivery was strongly associated with invasive Hib infection (OR 4.4), in keeping with the immunologic findings above.

Given imperfect protection by vaccination, other non-vaccine factors that may have contributed to disease risk in otherwise healthy children were explored. Living in a single parent household (OR 2.61) was linked with increased likelihood of invasive infection. In a surprising finding, a protective effect of exposure to other children outside the home in day care settings was observed, increasing with the number of children in care (OR (trend) 0.78). A further surprise was a protective effect of passive smoking (OR (trend) 0.55). Both of these latter two exposures have been associated with an increased likelihood of carriage of respiratory pathogens. Repeated colonisation with Hib in children who have been immunised may result in better persistence of vaccine-induced protection throughout childhood.

The medium to long-term impact of disruption of Hib transmission on maintenance of immunity in the population has been noted. The anticipated effect of these shifts in protection on carriage and disease will be explored in subsequent chapters, using epidemiological models of Hib infection and immunisation.

Chapter 4: Model Structure

4.0 Introduction: Questions to be asked of the model

An epidemiological model of Hib infection and immunisation was developed in order to better understand what key determinants might be involved in a resurgence of invasive Hib disease in the UK eight years after vaccine introduction, following a period of very low incidence. This framework was designed to allow examination of several key assumptions to do with the protection afforded by natural and vaccine induced immunity including:

- Duration of protection
- Degree of protection against acquisition of Hib carriage
- Degree of protection against progression of established carriage to invasive disease

Factors influencing transmission of the organism and subsequent maintenance of population immunity which could be explored included:

- Imposition of a vaccine known to block acquisition of Hib carriage
- The impact of assumptions regarding social mixing patterns between age groups on vaccine effectiveness post implementation

Issues of particular relevance to the isolated rise in disease observed in the UK included consideration of aspects of vaccine implementation unique to Britain:

- How might the 'catch-up' immunisation programme, which accompanied the Hib vaccine's introduction in the UK, have altered the observed effectiveness of the accelerated three dose primary infant schedule?
- How might the use of less immunogenic Hib combination vaccines, such as those used in the UK in 2000/2001, have contributed to the increase in Hib disease?

Based on an understanding of these interactions and their consequences for Hib disease over the past ten years, we wished to be able to ask:

- What are the potential public health benefits of longer term interventions such as
    - a less accelerated primary schedule?
    - addition of a routine booster dose?
    - intermittent catch up campaigns?

4.1 Model structure

The model is a fully age structured deterministic susceptible-infected-resistant-susceptible (SIRS) model with modifications, expressed as a set of partial differential equations. State variables are defined in Table 4.0, and flows between compartments are illustrated in Figure 4.0. These compartments may be broadly grouped into 'susceptible', 'infected' and 'resistant' states, as denoted by the shadings of green, yellow and blue, respectively. In order to address the questions defined in part 1 above, these categories have been further subdivided into biologically defined subgroups described below in Table 4.0.

To avoid the need to consider maternal immunity, all subjects enter the model at the age of 6 months, and progress through it until the age of 20.5 years, at which point they exit. Initial conditions reflect the population distribution of individuals who are susceptible to, infected with, or resistant to Hib infection in the pre vaccine era. At the time of introduction of immunisation, a fraction of each new birth cohort enters the vaccinated proportion, with the remainder continuing to be 'born' into the unvaccinated class. The distinction between these two groups remains throughout life. 'Catchup' immunisation of individuals not vaccinated at birth is simulated by transfer of the relevant proportion of subjects in the target birth cohorts from the unvaccinated susceptible and resistant states to the vaccinated high antibody resistant compartment. Those children who are infected at the time of immunisation move to the corresponding vaccinated infected class.

All of the 'infections' in the model refer to episodes of oropharyngeal carriage. Some of these infections may progress to invasive disease. Due to the relative rarity of such an event, invasive episodes are not explicitly described within the model structure, but calculated with a quasi-steady state approximation, based on the number of infections of various types at any point in time.

4.1.1 PreVaccination Condition

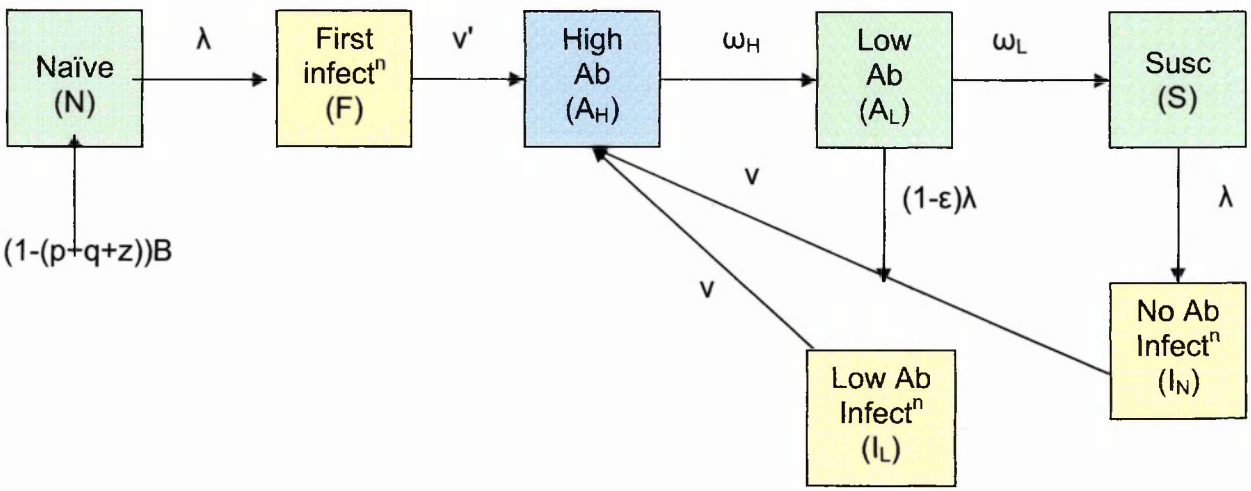
Mutually exclusive compartments represent the different epidemiological status of individuals in the population in relation to the infection. Arrows describe flows between these compartments. Individuals are born immunologically naïve (N), until they acquire their first Hib infection (F). The absence of any pre-existing immunity at the point of exposure to the organism means that this initial encounter is particularly prone to result in serious invasive disease. Following clearance of colonisation, individuals seroconvert and move to the high antibody (A_H) compartment. This state denotes a period of absolute resistance to reacquisition of infection. Over time, immunity wanes and individuals move into the low antibody (A_L) category. Here the presence of low level measurable immunity provides incomplete protection against infection, which may be so poor as to be considered absent. With the waning of antibody from low to undetectable levels, individuals are once again fully susceptible to acquisition (S). Infections acquired in the presence of low circulating antibody (I_L) are assumed to have a reduced likelihood of progressing to invasive disease. Those acquired in the absence of circulating antibody (I_N) have a slightly higher risk of becoming invasive, but there is still less chance of this occurring than with the first infection (F). With clearance of carriage from either the I_L or I_N state, immunity is stimulated to the protective (A_H) level and the cycle of waning immunity, reinfection and stimulation of immunity begins again.

Table 4.0: Definitions of State Variables

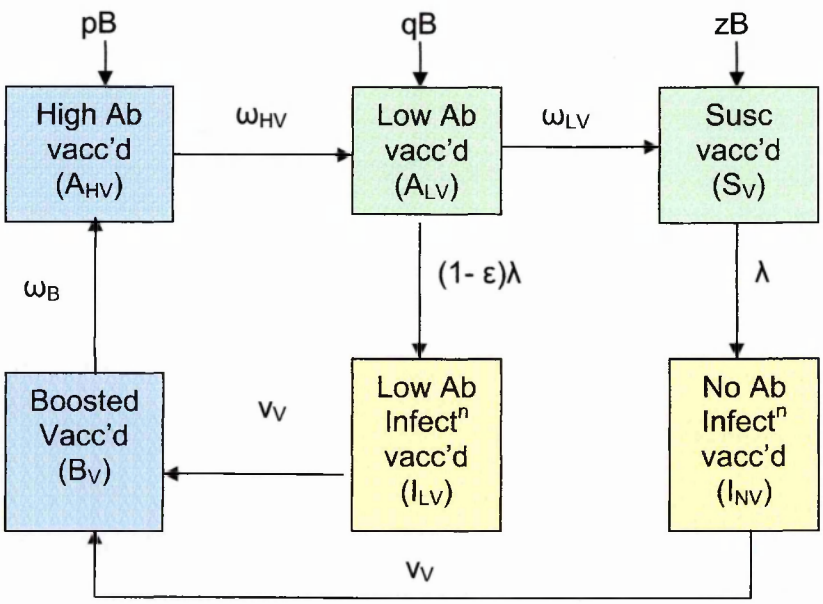
State Variable	*Symbol*	*Definition*
a. Unvaccinated Proportion		
Naïve	N	Unvaccinated individuals who have never been infected with Hib.
First infection	F	Unvaccinated individuals infected for the first time with Hib, who are highly vulnerable to progression to invasive disease.
High Antibody	A_H	Unvaccinated individuals who have cleared a recent Hib infection, with resultant development of specific immunity to a level sufficient to completely block reacquisition of carriage.
Low Antibody	A_L	Unvaccinated individuals whose immunity following Hib infection is still detectable, but has waned to a low level. Reacquisition of the organism is possible in this state, although the likelihood may be reduced.
Susceptible	S	Unvaccinated individuals whose immunity following Hib infection has waned to a level undetectable on routine serum testing. Without measurable antibody, these individuals are fully susceptible to acquisition of carriage.
Low Antibody Infection	I_L	Unvaccinated individuals who have become infected with Hib in the presence of low level serum antibodies. This level of immunity at the point of exposure is assumed to provide partial protection against invasive disease.
No Antibody Infection	I_N	Unvaccinated individuals who have become infected with Hib in the absence of any measurable specific immunity. Previous exposure to the organism has left some residual immunity, but this is assumed to provide less protection against progression to invasive disease than the low levels of antibody present in the I_L compartment above.
b. Vaccinated Proportion		
High Antibody Vaccinated	A_{HV}	Vaccinated individuals who have achieved high post immunisation antibody titres sufficient to protect them from acquisition of Hib.
Low Antibody Vaccinated	A_{LV}	Vaccinated individuals whose antibody titres have waned following immunisation, or were insufficiently high following immunisation, to completely protect them against acquisition. They may be partially protected by the low level of specific immunity measurable.
Susceptible Vaccinated	S_V	Vaccinated individuals whose antibody titres have waned to undetectable levels following immunisation, or made no apparent response to immunisation. Without measurable antibody, these individuals are fully susceptible to acquisition of carriage.
Low Antibody Infection Vaccinated	I_{LV}	Vaccinated individuals who have become infected with Hib in the presence of low level serum antibodies. This level of immunity at the point of exposure is assumed to provide partial protection against invasive disease.
No Antibody Infection Vaccinated	I_{NV}	Vaccinated individuals who have become infected with Hib in the absence of any measurable specific immunity. Previous vaccination has left some residual immunity, but this is assumed to provide less protection against progression to invasive disease than the low levels of antibody present in the I_{LV} compartment above.
Boosted Vaccinated	B_V	Vaccinated individuals who have cleared infection with Hib, with resultant development of very high ('boosted') levels of immunity sufficient to block acquisition of carriage.

Figure 4.0: Age Structured Model

a. Unvaccinated Proportion



b. Vaccinated Proportion



4.1.2 Vaccination

Immediately following primary vaccination, individuals enter the model with high (A_{HV}), low (A_{LV}) or undetectable (S_V) antibody levels. This bypassing of the immunologically naïve (N) state removes the risk of progression to invasive disease associated with the first ever infection (F) which is experienced by the unvaccinated class. The proportion in each immune category at the outset depends on the immunogenicity of vaccines in use at any given time. The degree of protection against acquisition in these three states is assumed to be identical to that experienced by unvaccinated individuals with high (A_H), low (A_L) or undetectable (S) immunity. As before, infections may arise in the face of low or unmeasurable antibody, in this instance denoted as I_{LV} and I_{NV} respectively. The same risks of progression to invasive disease are assumed as from the I_L and I_N conditions. Following recovery from infection, immunity is boosted to a higher level than would be seen in an unvaccinated population (B_V). From here, antibodies wane to the post immunisation level (A_{HV}), with this additional step making the duration of protection following an episode of natural exposure longer in individuals who have been vaccinated. With waning of immunity, the cycle of reacquisition and boosting of antibody levels can recur.

The model is expressed in the following series of partial differential equations:

$$\frac{\partial N}{\partial t} + \frac{\partial N}{\partial a} = [1-(p(t) + q(t) + z(t))] \times B - \lambda(a,t)N(a,t)$$

$$\frac{\partial F}{\partial t} + \frac{\partial F}{\partial a} = \lambda(a,t)N(a,t) - \upsilon'(a)F(a,t)$$

$$\frac{\partial A_H}{\partial t} + \frac{\partial A_H}{\partial a} = \upsilon'(a)F(a,t) + \upsilon(I_L(a,t) + I_N(a,t)) - \omega_H(a)A_H(a,t)$$

$$\frac{\partial A_L}{\partial t} + \frac{\partial A_L}{\partial a} = \omega_H(a)A_H(a,t) - \omega_L A_L(a,t) - (1-\varepsilon)\lambda(a,t)A_L(a,t)$$

$$\frac{\partial S}{\partial t} + \frac{\partial S}{\partial a} = \omega_L A_L(a,t) - \lambda(a,t)S(a,t)$$

$$\frac{\partial I_L}{\partial t} + \frac{\partial I_L}{\partial a} = (1-\varepsilon)\lambda(a,t)A_L(a,t) - \upsilon I_L(a,t)$$

$$\frac{\partial I_N}{\partial t} + \frac{\partial I_N}{\partial a} = \lambda(a,t)S(a,t) - \upsilon I_N(a,t)$$

$$\frac{\partial A_{HV}}{\partial t} + \frac{\partial A_{HV}}{\partial a} = p(t)B + \omega_B(a)B_V(a,t) - \omega_{HV}(a)A_{HV}(a,t)$$

$$\frac{\partial A_{LV}}{\partial t} + \frac{\partial A_{LV}}{\partial a} = q(t)B + \omega_{HV}(a)A_{HV}(a,t) - (\omega_{LV} + (1-\varepsilon)\lambda(a,t))A_{LV}(a,t)$$

$$\frac{\partial S_V}{\partial t} + \frac{\partial S_V}{\partial a} = z(t)B + \omega_{LV}A_{LV}(a,t) - \lambda(a,t)S_V(a,t)$$

$$\frac{\partial I_{LV}}{\partial t} + \frac{\partial I_{LV}}{\partial a} = (1-\varepsilon)\lambda(a,t)A_{LV}(a,t) - \upsilon_V I_{LV}(a,t)$$

$$\frac{\partial I_{NV}}{\partial t} + \frac{\partial I_{NV}}{\partial a} = \lambda(a,t)S_V(a,t) - \upsilon_V I_{NV}(a,t)$$

$$\frac{\partial B_V}{\partial t} + \frac{\partial B_V}{\partial a} = \upsilon_V(I_{LV}(a,t) + I_{NV}(a,t)) - \omega_B(a)B_V(a,t)$$

Chapter 5: Model Parameters

5.0 Introduction

The model has a high degree of structure, requiring extensive analysis of the published literature in order to define a range of parameter values. The interactions defined in the model provide a strong organising framework within which to examine this literature. Table 5.0 summarises the initial parameters estimated, and lists the chapter section in which each one is derived.

Table 5.0: Summary of Parameter Values

Model Parameter	Mean Value	Mean duration in class	Derived in Section
Demographic parameters			
Births per quarter, B	6 250		
Population size, N	500 000		
Proportions vaccinated, p, q, z	0-1 where $p+q+z \leq 1$		
Routine infant immunisation	p=0.83, q=0.07, z=0.02		5.1
Poorly immunogenic vaccine	p=0.63, q=0.19, z=0.10		"
Biological parameters			
Duration of carriage for first infection			
υ'	5	2.4 months	5.2
Duration of carriage for subsequent infections			
υ (unvaccinated)	5	2.4 months	5.2
υ_V (vaccinated)	5	2.4 months	5.2
Duration of immunity			
Unvaccinated			
ω_{H1} (0.5-1.99 years)	6.59	0.15 years	5.3.2, 5.5
ω_{H2} (2-4.99 years)	0.68	1.46 years	"
ω_{H3} (5-10.99 years)	0.47	2.13 years	"
ω_{H4} (11-20.5 years)	0.49	2.05 years	"
ω_L (all ages)	0.09	11.0 years	"
Vaccinated			
ω_{HV1} (0.5-0.99 years)	0.81	1.23 years	5.3.3
ω_{HV2} (1-1.49 years)	0.56	1.77 years	"
ω_{HV3} (1.5-1.99 years)	0.48	2.08 years	"
ω_{HV4} (2-20.5 years)	0.40	2.48 years	"
ω_{LV} (all ages)	0.69	1.46 years	"
Duration of immunity following re-exposure to Hib in vaccinated individuals			
ω_{B1} (0.5-0.99 years)	0.43	2.30 years	5.3.4
ω_{B2} (1-1.49 years)	0.56	1.77 years	"
ω_{B3} (1.5-1.99 years)	0.69	1.46 years	"
ω_{B4} (2-20.5 years)	0.94	1.07 years	"

Model Parameter	Mean Value	Mean duration in class	Derived in Section
Relative protection against acquisition from low antibody state			
ε	0-1		5.4
Force of infection, λ			
λ_1 (0.5-1.99 years)	0.45		5.5
λ_2 (2-4.99 years)	0.22		"
λ_3 (5-10.99 years)	0.40		"
λ_4 (11-20.5 years)	0.55		"
Rate of progression to invasive disease, δ			
δ_1 (0.5-0.99 years)	0.0127		5.6
δ_2 (1.0-1.99 years)	0.0042		"
δ_3 (2.0-2.99 years)	0.0037		"
δ_4 (3.0-3.99 years)	0.0030		"
δ_5 (4.0-4.99 years)	0.0016		"
δ_6 (5.0-20.5 years)	0.00006		"
Protection against progression against invasive disease afforded by low levels of measurable antibody, θ			
Θ	0		5.6
Protection against progression against invasive disease afforded by previous natural or vaccine exposure to Hib in the absence of measurable antibody, μ			
M	0		5.6

5.1 Proportions Vaccinated (p, q, z)

5.1.1 Routine Infant Immunisation

Literature Review

The proportions of individuals entering the model in the vaccinated (p+q+z) and unvaccinated [1-(p+q+z)] categories were derived from COVER data published quarterly by the HPA CDSC (http://www.hpa.org.uk/cdr/archive04/immunisation04.htm). Figures for complete (three doses) Hib vaccine coverage by 12 months were used. These data are illustrated in Figure 2.1 to demonstrate the relative consistency of coverage over time, compared with receipt of the first dose of the combined measles, mumps and rubella vaccine by 24 months of age which has fallen in recent years due to adverse publicity.

As well as denoting the proportion of the population who were immunised, the parameters p, q and z represent the quality of vaccine 'take' achieved at different times during the Hib immunisation campaign. This is of particular importance in considering the likely impact of introducing less immunogenic preparations, such as Infanrix-Hib – a Hib vaccine conjugated with tetanus toxoid, formulated in combination with acellular pertussis, diphtheria and tetanus (DTaP-Hib). This vaccine was in widespread use in the UK in 2000/2001. At that time, approximately one third of infants received three doses of Infanrix-Hib in their primary infant schedule, and another third received a mixture of this and the corresponding whole cell pertussis containing combination vaccine DTwP-Hib (McVernon J et al 2003a). Evidence for the reduced efficacy of this vaccine, in association with its observed poor immunogenicity, has been outlined in Section 3.1.2 of Chapter 3.

In the model, immunisation may be associated with induction of high, low or undetectable antibody titres which result in a different degree and duration of protection against acquisition of infection. Phase II clinical vaccine trials routinely report immunogenicity results according to the cutoffs of 1.0 and 0.15 μg/ml. These antibody titres were associated with long and short term protection against Hib disease, respectively, in the era before availability of conjugate vaccines (Kayhty H et al 1983). In the absence of any other satisfactorily defined serological correlate of protection, these values have been arbitrarily used to distinguish between the compartments A_{HV}, A_{LV} and S_V. Vaccinees who fail to seroconvert following primary immunisation (<0.15 μg/ml) may later demonstrate evidence of immunologic memory on boosting (Goldblatt D et al 1999) and so for the purposes of the model were initially considered to enter the vaccinated susceptible category rather than being completely naïve. This assumption was later explored in the sensitivity analysis, by varying the proportion of children who were primary vaccine failures and entered the fully naïve class (N).

Proportions of subjects achieving protective thresholds following immunisation with different vaccines administered according to the UK infant schedule are summarised in Table 5.1. Quite considerable variation is seen between studies. It should be noted that the combination vaccine in the Poolman study was a hexavalent preparation not available for routine use in the UK which also included hepatitis B and injectable polio vaccines.

Table 5.1: Immunogenicity of different Hib vaccine schedules in infancy

Reference	Vaccines and Schedule	Number of subjects	% >1.0 μg/ml	% 0.15-1 μg/ml	% <0.15 μg/ml
Heath PT 2000	DTwP-Hib 2,3,4 mo	105	88%	11%	1%
Goldblatt 1998	DTwP-Hib 2,3,4 mo	516	93%	5%	2%
Bell F et al 1998	DTaP-Hib 2,3,4 mo	251	23%	48%	29%
Poolman J et al 2001	DTaP-Hib 2,3,4 mo	172	75%	24.4%	0.6%
Goldblatt et al 1999	DTaP-Hib 2,3,4 mo	122	57%	38.1%	4.9%

Parameter Estimation

Taking an average of the seroconversion rates from the Heath and Goldblatt studies above, we may assume that following primary immunisation with DTwP-Hib, 90% of children achieve high antibody titres (>1.0 μg/ml), 8% have low antibody (0.15-1.0 μg/ml) and 2% undetectable antibody (<0.15 μg/ml). If population immunisation coverage is 92%, the following routine values for p, q and z are obtained:

$$p = 0.83; \qquad q = 0.07, \qquad z = 0.02$$

In 2000/2001 one third of children (30%) received three doses of poorly immunogenic Hib vaccine (DTaP-Hib), while the remainder (62%) were given at least one dose of DTwP-Hib. The worst case scenario in terms of seroconversion rates is assumed from Bell's data to derive the following values for p, q and z over this two year period:

Entry into model	A_{HV}	A_{LV}	S_V
3 doses DTaP-Hib	0.23 ×0.30	0.48 ×0.30	0.29 ×0.30
Any DTwP-Hib	0.90 ×0.62	0.08 ×0.62	0.02 ×0.62
Immunisation parameter	p = 0.63	q = 0.19	z = 0.10

5.1.2 Catch-up Immunisation Campaign

Literature Review

The catch-up immunisation campaign that accompanied Hib vaccine introduction over the first year of its use has been described in Section 2.1 of Chapter 2. Figure 2.0 shows quarterly immunisation coverage by birth cohort in the North Thames region, which is the best data available on which to base estimates of national catch-up vaccine uptake (O'Brien H 1994). .

Table 5.2 below shows the excellent immunogenicity of a single dose of the CRM_{197} conjugate Hib vaccine (HbOC) in children aged 12 months and older, with 98-99% achieving Hib antibody titres >1.0 μg/ml. This formulation was widely used for catch-up immunisation in the UK (Section 2.3, Table 2.0). On the basis of these data, all susceptible and resistant children immunised in the catch-up were assumed to transfer directly into the A_{HV} compartment following immunisation in the model simulation.

Children who were infected at the time of immunisation were transferred to the corresponding low or no antibody immunised infected compartment (ie $I_L \rightarrow I_{LV}$, $I_N \rightarrow I_{NV}$). This was based on a study in toddlers which showed that conjugate

vaccination did not clear established carriage over a subsequent six week period, in spite of evidence of seroconversion (Barbour ML et al 1995).

Table 5.2: Immunogenicity of different Hib vaccine schedules in toddlers

Reference	Vaccines and Schedule	Number of subjects	% >1.0 µg/ml
Rothstein 1991	HbOC 12-15 mo	14	98%
Madore 1990b	HbOC 15-23 mo	171	99%

Parameter Estimation

Table 5.3 shows the calculations used to parameterise the catch-up campaign over the first year following vaccine introduction at time t=5. Quarterly coverage data provided the cumulative proportion in the four relevant birth cohorts immunised by the end of each three month period. From this information, the fraction of the remaining unvaccinated population transferred to the immunised class was calculated at each time point.

Table 5.3: Parameterisation of the catch-up immunisation campaign 1992-1993

Time	Age Classes Involved	Cumulative Coverage	Proportion of Remainder Transferred	Equations Describing Transfers Between Compartments
1989 Birth Cohort				
5	12, 13, 14, 15	0.10	$0.10 \div 1 = 0.10$	$A_{HV}=0.10\times(N+F+A_H+A_L+S)$ $I_{NV}=0.10\times I_N$; $I_{LV}=0.10\times I_L$
5.25	13, 14, 15, 16	0.25	$0.15 \div 0.90 = 0.167$	$A_{HV}=0.167\times(N+F+A_H+A_L+S)$ $I_{NV}=0.167\times I_N$; $I_{LV}=0.167\times I_L$
5.5	14, 15, 16, 17	0.30	$0.05 \div 0.75 = 0.067$	$A_{HV}=0.067\times(N+F+A_H+A_L+S)$ $I_{NV}=0.067\times I_N$; $I_{LV}=0.067\times I_L$
5.75	15, 16, 17, 18	0.34	$0.04 \div 0.70 = 0.057$	$A_{HV}=0.057\times(N+F+A_H+A_L+S)$ $I_{NV}=0.057\times I_N$; $I_{LV}=0.057\times I_L$
1990 Birth Cohort				
5	8, 9, 10, 11	0.25	$0.25 \div 1 = 0.25$	$A_{HV}=0.25\times(N+F+A_H+A_L+S)$ $I_{NV}=0.25\times I_N$; $I_{LV}=0.25\times I_L$
5.25	9, 10, 11, 12	0.40	$0.15 \div 0.75 = 0.20$	$A_{HV}=0.20\times(N+F+A_H+A_L+S)$ $I_{NV}=0.20\times I_N$; $I_{LV}=0.20\times I_L$
5.5	10, 11, 12, 13	0.60	$0.20 \div 0.60 = 0.33$	$A_{HV}=0.33\times(N+F+A_H+A_L+S)$ $I_{NV}=0.33\times I_N$; $I_{LV}=0.33\times I_L$
5.75	11, 12, 13, 14	0.77	$0.17 \div 0.40 = 0.425$	$A_{HV}=0.425\times(N+F+A_H+A_L+S)$ $I_{NV}=0.425\times I_N$; $I_{LV}=0.425\times I_L$
1991 Birth Cohort				
5	4, 5, 6, 7	0.20	$0.20 \div 1 = 0.20$	$A_{HV}=0.20\times(N+F+A_H+A_L+S)$ $I_{NV}=0.20\times I_N$; $I_{LV}=0.20\times I_L$
5.25	5, 6, 7, 8	0.60	$0.40 \div 0.80 = 0.50$	$A_{HV}=0.50\times(N+F+A_H+A_L+S)$ $I_{NV}=0.50\times I_N$; $I_{LV}=0.50\times I_L$
5.5	6, 7, 8, 9	0.75	$0.15 \div 0.40 = 0.375$	$A_{HV}=0.375\times(N+F+A_H+A_L+S)$ $I_{NV}=0.375\times I_N$; $I_{LV}=0.375\times I_L$
5.75	7, 8, 9, 10	0.87	$0.12 \div 0.25 = 0.48$	$A_{HV}=0.48\times(N+F+A_H+A_L+S)$ $I_{NV}=0.48\times I_N$; $I_{LV}=0.48\times I_L$
1992 Birth Cohort				
5	1, 2, 3	0.50	$0.50 \div 1 = 0.50$	$A_{HV}=0.50\times(N+F+A_H+A_L+S)$ $I_{NV}=0.50\times I_N$; $I_{LV}=0.50\times I_L$
5.25	2, 3, 4	0.75	$0.25 \div 0.50 = 0.50$	$A_{HV}=0.50\times(N+F+A_H+A_L+S)$ $I_{NV}=0.50\times I_N$; $I_{LV}=0.50\times I_L$
5.5	3, 4, 5	0.80	$0.05 \div 0.25 = 0.20$	$A_{HV}=0.20\times(N+F+A_H+A_L+S)$ $I_{NV}=0.20\times I_N$; $I_{LV}=0.20\times I_L$
5.75	4, 5, 6	0.89	$0.09 \div 0.20 = 0.45$	$A_{HV}=0.45\times(N+F+A_H+A_L+S)$ $I_{NV}=0.45\times I_N$; $I_{LV}=0.45\times I_L$

5.2 Duration of Carriage (v', v, v_Y)

Literature Review

Only a few longitudinal studies of Hib carriage in infancy and early childhood have been published. Turk observed infants entering a Jamaican orphanage, many of whom acquired Hib following approximately 6 weeks in the institution. The interval between swabbing visits was variable, and many infants were placed outside the nursery before clearance of carriage could be documented, making it difficult to give a precise estimate of the duration of colonisation, although 'no child was observed to carry a type b strain for more than 3 months' (Turk 1963). Table 5.4 is taken from his 1963 publication.

Marina Barbour followed immunised and unimmunised UK infants in the early 1990s over the first year of life, swabbing at 6, 9 and 12 months of age. Hib was isolated at least once from 1.5% of vaccinees and 6.3% of controls over this period. Those who had been vaccinated tended to acquire infections later, at 9 months of age, compared with 6 months of age in the unvaccinated group. Of 91 swabs positive for Hib in the study, 20 were second or third isolates from the same subject. It is not clear, however, how many of these repeat cultures were from the infants studied, and how many were from a slightly older group of children who took part in a further substudy, making estimation of carriage duration difficult. This latter group comprised 17 known Hib carriers of an age eligible to participate in the government's catch-up vaccine campaign, half of whom were immunised with a single dose of conjugate vaccine. They were then swabbed weekly for 6 weeks, at the end of which time 14 remained carriers. It would therefore seem reasonable to assume that very few babies carried on more than one occasion (Barbour ML et al 1995).

Table 5.4: The Orphan Home Nursery, August 1960 to March 1961 (from Turk DC 1963)

Ref No	Age (mo)	Sex	Date admitted	Swab Date and Result							
				29th Aug	31st Aug	27th Sep	12th Oct	27th Oct	28th Nov	26th Jan	2nd Mar
1	7	F	April	b	b	+	b	b	b +	.	.
2	12	F	April	b	+	(b)	.	.	(b)	.	.
3	7	M	April	b	b	b	b	b+	+	+	+
4	8	F	April	b	b	+	b	b+	b	.	.
5	4	F	May	b	b	b	b	.	.	.	.
6	9	M	June	-	-	+	b	.	(b)	.	.
7	2	F	June	+	+	b	b	b+	b	b	.
8	5	M	July	b	b	b	b	b	b	+	+
9	5	F	July	b	b	+	+	+	b	+	+
10	9	M	July	+	+	b+	+	+	+	b+	+
11	4	M	8 Sept	.	.	-	-	-	b	b	-
12	3	F	9 Sept	.	.	-	+	-	b+	.	.
13	?1	M	12 Sept	.	.	-	-	+	+	+	+
14	4	M	14 Sept	.	.	-	b	?	b	+	+
15	8	F	*	.	.	.	?	b+	b+	+	+
16	?8	M	*	.	.	.	-	-	+	b	b+
17	3	M	21 Nov	.	.	.	.	.	-	+	b+
18	12	M	30 Nov	.	.	.	.	.	.	+	+
19	5	F	24 Dec	.	.	.	.	.	.	+	+

Legend to symbols:

- no *H Influenzae* grown

+ non-capsulate strains grown

b type b grown

b+ type b and non-capsulate strains grown

(b) type b grown but child was no longer in the nursery

? cultures overgrown by *Proteus* spp

. not present on this date

* in the home before period of observation began, but absent during August, early September

While these infant studies are a source of many valuable observations, they do not provide sufficient information upon which to base a precise estimate of the rate of clearance of colonisation. An average carriage duration of 2.4 months was noted in toddlers in a nursery in the United States observed over one year (Murphy TV et al 1985). Such a figure is not inconsistent with the data from the studies in young babies, and so for the purposes of the model this value for the time spent in the infected class has been assumed to be age independent.

A previous estimate of carriage duration of 5 months incorporated in a mathematical model of Hib infection (Coen PG et al 1998) was derived from a family study of children recovering from Hib disease. This work was conducted in the era before routine use of Rifampicin prophylaxis to eradicate colonisation in disease contacts (Michaels RH et al 1977). It should be noted that only children with an existing carrier in the family to which they were returning were followed over a year in this original study, meaning that the estimate most likely incorporates episodes of reacquisition in children too young to mount an effective immune response. In support of this notion, the authors of the study noted that only 2 of 15 patients discharged to homes without carriers remained colonised after a period of 'several weeks'. It is thus most likely that the average rate of clearance of carriage is more rapid than would be estimated from these data.

Parameter estimation

If the average time spent in the infected compartment is assumed to be 2.4 months, the annual rate of clearance is 12months ÷ 2.4 months = 5 episodes potentially cleared per year.

5.3 Duration of specific immunity ($\omega_H(a)$, ω_L, $\omega_{HV}(a)$, ω_{LV})

5.3.1 Definition of the thresholds between high, low and no measurable antibody compartments

Literature Review

Individuals with high (A_H, A_{HV}), low (A_L, A_{LV}) or undetectable (S, S_V) levels of serum antibody are assumed to experience different degrees of protection against acquisition of Hib carriage, and progression to invasive disease. In order to be able to parameterise the rate of movement between compartments, threshold antibody titres defining the divisions between these states must be estimated.

Prior to the use of conjugate vaccines, an attempt was made to correlate observed anti PRP antibody titres in polysaccharide immunised and passively protected individuals with development of invasive disease (Robbins JB et al 1996). In addition, seroepidemiologic studies were correlated with the observed age dependent incidence of Hib in whole populations (Kayhty H et al 1983a). The short and long term correlates of protection of 0.15 μg/ml and 1.0 μg/ml respectively were proposed.

Animal data obtained in an infant rat model have demonstrated reduced replication of Hib at the mucosa in the presence of a serum antibody concentration of 1.0 μg/ml (van Alphen L et al 1996). Much higher serum Hib antibody concentrations were required to block acquisition altogether in this model, ranging from 7-20 μg/ml (van Alphen L et al 1996, Kauppi-Korkeila M et al 1996). Challenge trials of this nature have not been conducted in humans, forcing us to look to studies where immune status is known before spontaneous acquisition of carriage in the community is documented.

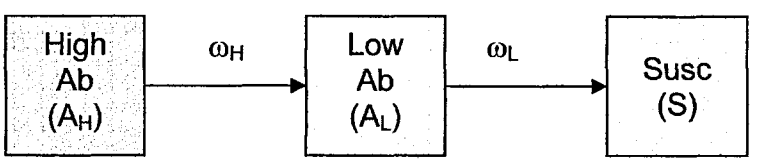
Phase II vaccine trials combined with carriage studies are a source of this kind of information. One such study, conducted in the Dominican Republic, has estimated the level of serum antibody required to block colonisation in children to be 5.0 μg/ml. This figure was based on correlation of antibody titres following conjugate immunisation with demonstration of carriage several months later (Fernandez J et al 2000). Larger longitudinal carriage studies of groups of vaccinated individuals suggest that protection against acquisition may last for longer following primary immunisation than such a high threshold would imply. Vaccinated English infants studied in a family setting over the first year of life showed an 80% reduction in Hib colonisation compared with their unvaccinated counterparts (Barbour ML et al 1995). The expected Hib antibody GMC one month following primary immunisation at a 2, 3 and 4 month accelerated schedule in British infants administered single antigen Hib vaccine is 4.60 μg/ml (95% confidence intervals 3.51, 6.03) (Heath PT et al 2000a). At the same schedule, the response to Hib combined with diphtheria, tetanus and whole cell pertussis is similar at 6.23 μg/ml (5.53, 7.01) (Goldblatt D et al 1998c). If an antibody threshold of 5.0 μg/ml is believed to correlate with protection, this would have lasted for no more than a matter of weeks following immunisation as titres wane rapidly following the primary immune response. A similarly high degree of protection (81%) over the first year following vaccination was noted in toddlers attending nursery in the United States, in an analysis which matched immunised and unimmunised children on the basis of known exposure to a Hib carrier (Murphy TV et al 1993).

Parameter Estimation

In the absence of an adequately established correlate of protection, arbitrary cut-offs have been set in order to define the boundaries between state variables in the model. A

titre of ≥1.0 μg/ml has been assumed to protect against acquisition, and is thus defined as the lower limit of antibody required for individuals to remain in the high antibody unvaccinated (A_H) and high antibody vaccinated (A_{HV}) compartments. Those with a low but measurable antibody titre, between 0.15 and 1.0 μg/ml, remain in the low antibody unvaccinated (A_L) and vaccinated (A_{LV}) compartments. Once antibodies wane below detectable levels, individuals move into the susceptible categories S or S_V, depending on immunisation status. The justification for these choices is based in part on the observation of 80% protection against carriage acquisition in the year following immunisation, as described above. The choice is also partly dictated by convenience, as immunogenicity studies are routinely reported in relation to these pre-defined thresholds, making comparison with observed seroepidemiologic and vaccine immunogenicity data easier.

5.3.2 Duration of natural immunity ($\omega_H(a)$, ω_L)



Literature Review

These parameters describe the critical interactions between exposure to Hib and immunity. In an ideal epidemiological world, one would have access to large datasets describing longitudinal observation of carriage episodes in individuals. These studies would additionally outline the relationship between pre-existing immunity and the likelihood of carriage acquisition, and the resulting effects on immunity of established carriage in both immunised and unimmunised individuals. Unfortunately, with the

exception of a few limited observations, these data do not exist. One must therefore consider what information can be gleaned from the available literature. Trends observed in small studies of carriage and immunity may be extended with data derived from immunisation trials and population level observations of the development of natural immunity with age in order to make sensible estimates of the duration of protection associated with a specific antibody response. These may later be subjected to sensitivity analyses within the spectrum of uncertainty inherent within the framework of such limited evidence.

Family studies conducted in households with a child recently recovered from invasive Hib infection have confirmed that acquisition of carriage, at least above the age of 18-24 months, is temporally associated with rising antibody titres in hosts, which subsequently fall over several months (Michaels RH et al 1977). These antibodies do not necessarily clear carriage. Barbour extended these observations to documented episodes of carriage 3-4 years following infant immunisation. Carriage was associated with a higher antibody response in children previously immunised with three doses of conjugate vaccine in infancy [Hib GMC 65.6 (95% CI 27.1, 102.8)] compared with those who had not been immunised [Hib GMC 3.51 (0.2, 31.2)]. The corresponding density of oropharyngeal colonisation, estimated using a semi-quantitative method of colony counting on an antiserum agar plate, was also lower in the vaccinated group (Barbour ML et al 1993).

Although these two studies give useful qualitative insights into the immunobiology of Hib infections, their design does not allow satisfactory estimation of the magnitude of the age dependent specific antibody response to Hib carriage. A long established surrogate for natural exposure, widely used by immunologists and vaccinologists alike, has been measurement of seroconversion following administration of the capsular

polysaccharide vaccine PRP. Age dependent maturation of immune responsiveness to the isolated polysaccharide mirrors that observed following exposure to the whole organism, justifying its use as a proxy marker (Robbins JB et al 1996). Studies of the response to a dose of PRP vaccine administered at different ages give us an estimate of the likely magnitude of the age specific immune response to carriage. These studies also provide some limited data on the rate of decline of antibody following PRP immunisation.

One major limitation of the use of PRP as a model of natural exposure is the absence of additional protein antigens in the vaccine which are present on the surface of the organism. Individuals convalescing from invasive Hib infection demonstrate an immune response to these antigens (Johnson PDR et al 1993). Their lack of correlation with bactericidal activity in serum, and lack of protective efficacy in animal models of infection (Srikumar R et al 1992), led to their abandonment as potential vaccine candidates. They may, however, have some accessory role in the immune response which is deficient in the PRP vaccine. There is a further problem with estimating waning rates by comparing antibody concentrations established following polysaccharide immunisation with those measured in the same individuals months or years later. It is highly likely that the young children involved in such studies will have had one or more episodes of exposure to Hib through carriage of the organism in the intervening period. Additional stimulation of the specific immune response by repeated colonisation may inflate the estimated duration of immunity following a single measured exposure. Key features of studies of polysaccharide immunisation are summarised below in order to draw general conclusions about the nature of development of the host response to polysaccharides, with these caveats in mind.

Helena Kayhty's group has published two papers on the age dependent immune response to polysaccharide vaccination, the first studying infants and children from 3 months to 6 years of age (Makela PH et al 1977), the second assessing vaccine immunogenicity in adult women with a mean age of 32 years (Makela O et al 1987). Results from these two sources are pooled in Table 5.5 below and represented graphically in Figure 5.1.

Table 5.5: Hib antibody titres 1 month post vaccination with PRP polysaccharide vaccine expressed as GMCs with 95% confidence intervals

Age at vaccination	Age in years	Number per group	Hib GMC (μg/ml)	Lower 95%CI	Upper 95%CI
3-5 mo	0.33	11	0.1	0.1	0.11
6-11 mo	0.71	40	0.25	0.17	0.37
12-17 mo	1.21	30	0.66	0.42	1.05
18-23 mo	1.71	26	3.17	1.9	5.3
24-35 mo	2.46	68	6.96	5.36	9.25
36-47 mo	3.46	52	9.04	6.82	11.98
48-71 mo	4.17	119	11.59	10.05	13.35
Adult	32.00	25	22.5	13.8	36.8

Figure 5.1: Post polysaccharide immunisation Hib antibody GMC by age

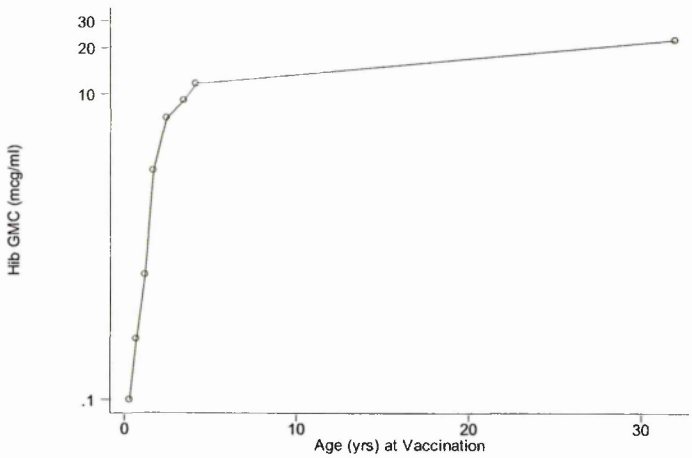


Figure 5.2 below details individual observations from another study conducted by this group of the rate of decline in antibody titres following primary and booster immunisation with the polysaccharide vaccine at different ages. While no specific numerical data is provided in the paper to correspond with these figures, it can be seen that the decay curves are parallel for all observations following a documented response to immunisation. If there is a minimum threshold for protection, it would thus seem logical that older children are protected for longer against re-acquisition, not because antibodies wane more slowly, but because they wane from a higher peak response.

Figure 5.2: Serum antibody decay curves following immunisation with Hib polysaccharide at different ages (from Kayhty H et al 1984)

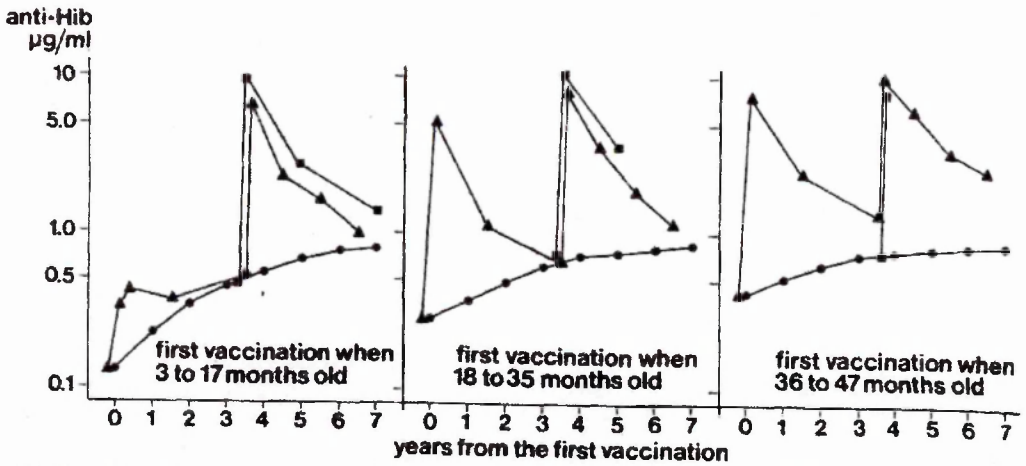


Fig 5. Kinetics of serum antibody responses after vaccination with one, two, or three doses of *Haemophilus influenzae* type b capsular polysaccharide (Hib) vaccine in different age groups. First dose was given at time 0, and children who were then younger than 18 months received a second dose 3 months later. A further dose was given 3½ years after the first one. Serum antibody levels were followed for 7 years in children immunized according to this schedule (solid triangle), and levels are compared with those in unimmunized children (solid circle) and those in children who received their first vaccine dose at indicated age (solid square).

Parameter Estimation

Given the limitations of the measured data above, an ordinary differential equation model was developed in Excel in order to estimate the duration of protection in unimmunised individuals of different ages following an episode of Hib carriage. Transitions between the compartments were calculated using a modified Euler approximation. The model is described by the following series of ordinary differential equations:

$$\frac{dN}{da} = -\lambda(a)N$$

$$\frac{dF}{da} = \lambda(a)N - \upsilon'(a)F$$

$$\frac{dA_H}{da} = \upsilon'(a)F + \upsilon[I_L + I_N] - \omega_H(a)A_H$$

$$\frac{dA_L}{da} = \omega_H(a)A_H - \omega_L A_L - (1-\varepsilon)\lambda(a)A_L$$

$$\frac{dS}{da} = \omega l A_L - \lambda(a)S$$

$$\frac{dI_L}{da} = (1-\varepsilon)\lambda(a)A_L - \upsilon I_L$$

$$\frac{dI_N}{da} = \lambda(a)S - \upsilon I_N$$

Model outputs were compared with cross sectional population observations of the prevalence of Hib carriage and immunity using a log likelihood method, as follows:

$$Deviance = 2 \times \left[(Observed\ Positive \times \ln (Model\ Positive \times Denominator)) + ((Denominator - Observed\ Positive) \times \ln ((1 - Model\ Positive) \times Denominator)) \right]$$

Deviance between observed and expected estimates was summed across age classes for both carriage and seroepidemiology data, and minimised by simultaneously varying the estimates of duration of high level immunity ($\omega_H(a)$) , low level immunity (ω_L) and the force of infection ($\lambda(a)$) across all four age classes while keeping all other parameters fixed.

Two large population based studies of Hib carriage were conducted prior to the use of widespread vaccination in developed countries. The first was carried out in children attending Health Department clinics and paediatricians' offices in the United States (Michaels RH et al 1976). While it is noted that the overall incidence of invasive Hib disease in the United States was almost twice that observed in the United Kingdom (Peltola H 2000), this study was unrivalled in its scope, sampling 1,110 healthy children ranging in age from birth to 16 years. Michaels and Norden optimised specimen transport and plating techniques for the purposes of this study, and noted that 'approximately two-thirds of the carriers were colonized at an intensity too low to be detected by standard laboratory techniques'. The only similar UK based study was conducted in Wales in the late 1980s, but studied children only up to 72 months of age,

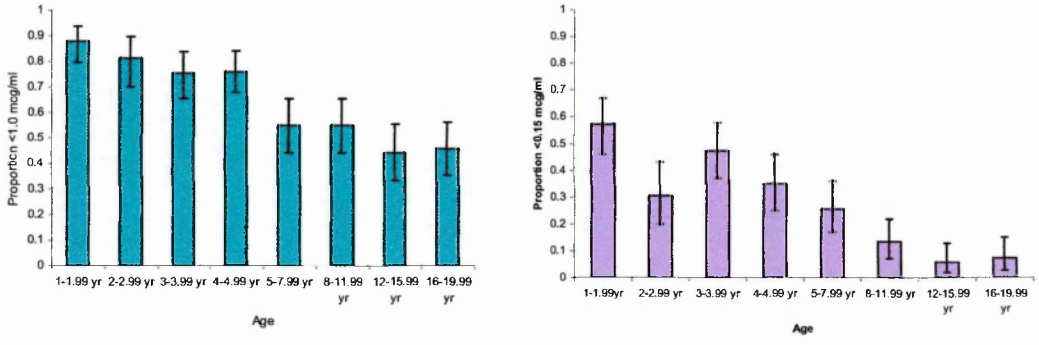
and employed less sensitive microbiological techniques (Howard AJ et al 1988). The population sample included child welfare clinics and primary school reception classes. These studies were discussed in Section 1.5 of Chapter 1, and their findings illustrated in Figure 1.1a) and 1.1b). For the purpose of model parameterisation, the larger dataset was used, bearing in mind the limitations of its source in another population.

Previous authors (Coen PG et al 1998) have utilised pooled data from many carriage studies spanning a range of countries in order to parameterise models of Hib infection. The difficulty in pooling such observations is firstly the range of microbiological methods used, which are recognised to have very different sensitivities as outlined above. In addition, the population from which samples are taken is of the utmost importance. In institutionally based studies in day care nurseries and schools, known carriers are likely to be in close contact with other susceptibles, leading to clusters of infected children in the sample. Comparison of such carriage estimates over time may be most informative, but may overestimate the true prevalence of colonisation in the general public. Similarly, misinterpretation of family based data can lead to similar errors.

The seroepidemiology data used to parameterise the model were taken from a study of changing UK population immunity to Hib over time conducted by the HPA (Trotter CL et al 2003b), discussed in detail earlier in Section 2.6.2. Data from 1990/1991 were collected before the introduction of Hib vaccination in the UK and are shown in Figure 5.3. 95% confidence intervals of estimates of the proportion of the population without protective antibody levels by age are represented by error bars. These are shown in relation to the two antibody thresholds used in the model of 1.0 μg/ml and 0.15 μg/ml.

Figure 5.3: Age distribution of prevalence of Hib antibody titres, United Kingdom, 1990/1991 (from Trotter CL et al 2003b)

a) <1.0 μg/ml **b) <0.15 μg/ml**



Optimal model fit to the observed data is shown below in Figures 5.4 (carriage) and 5.5 (seroepidemiology). Of particular interest is the higher than observed carriage rate required to explain population immunity under two years of age. Some or all of the exposures resulting in antibody formation at this age may be to cross reactive antigens and not Hib itself, which may in part explain the discrepancy.

Figure 5.4: Model fit to population carriage data (95% CI refer to observed dataset)

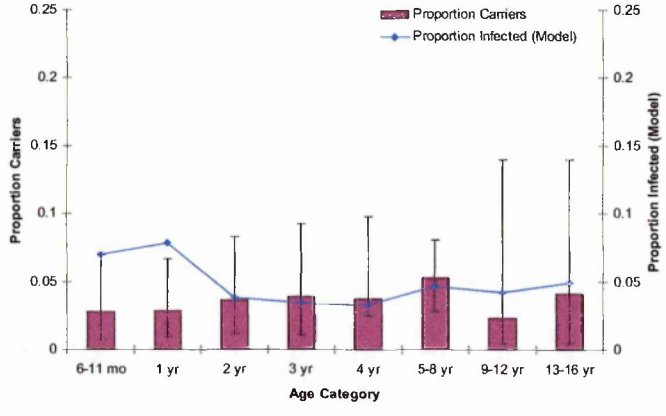
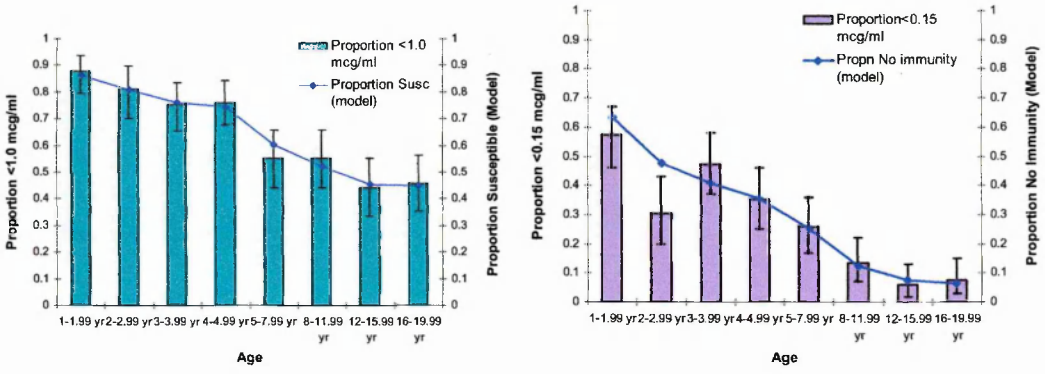


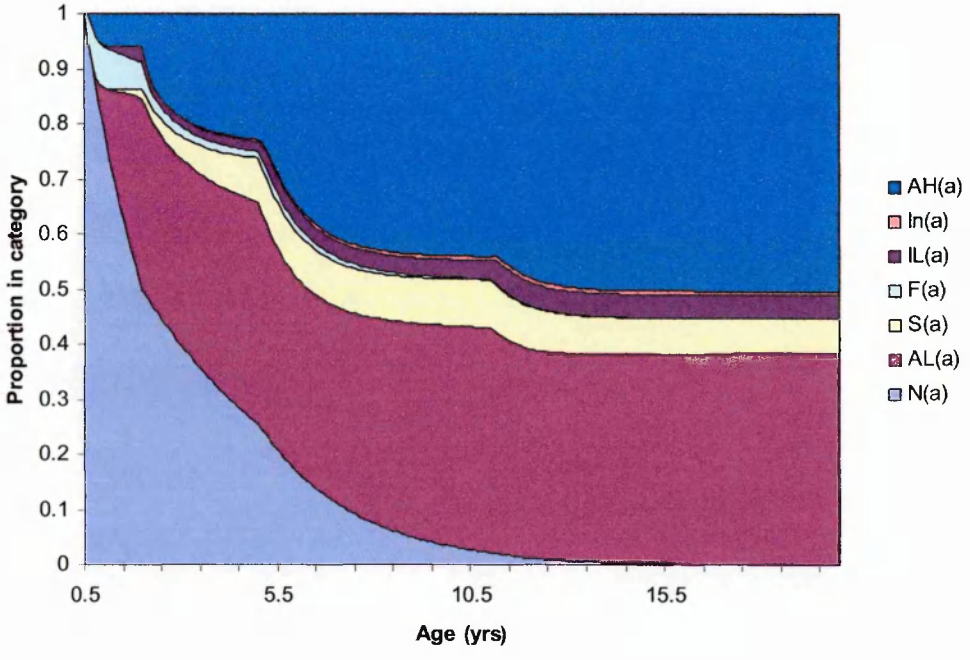
Figure 5.5: Model fit to population seroprevalence data (95% CI refer to observed dataset)

(i) Hib antibody titres <1.0 μg/ml **(ii) Hib antibody titres <0.15 μg/ml**



Distribution of individuals between compartments at equilibrium according to this fitted model is shown below in Figure 5.6.

Figure 5.6: Proportional age distribution of the population at equilibrium in the Excel model

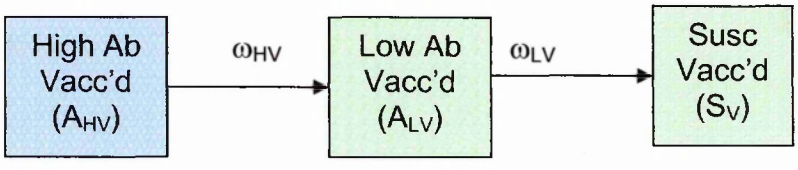


The calculated parameter values for $\omega_H(a)$ derived from this model were as follows:

Table 5.6: Duration of naturally acquired specific immunity against Hib

Age	Parameter Estimated	Mean Value	Mean Time to Waning
0.5-1.99 years	ω_{H1}	6.59	0.15 years
2-4.99 years	ω_{H2}	0.68	1.46 years
5-10.99 years	ω_{H3}	0.47	2.13 years
11-20.5 years	ω_{H4}	0.49	2.05 years
All ages	ω_L	0.09	11.0 years

5.3.3 Duration of immunity following conjugate vaccination ($\omega_{HV}(a)$, ω_{LV})



Literature Review

Estimation of the magnitude of the response to conjugate vaccines in children of different ages is readily available in the published literature. These address immunisation using a range of different conjugate vaccine formulations and schedules. Data are also available detailing the waning of the specific antibody response following immunisation at different ages.

Studies of the immunogenicity of the conjugate vaccines used in the UK, administered according to the routine infant immunisation and catch-up schedules, are summarised in Table 5.7 below. Data are also included from one Finnish study which compared the

antibody responses of adult women to Hib polysaccharide and an early diphtheria toxoid based Hib conjugate vaccine (PRP-D). Results in this last study are shown from 14 days post immunisation. In the original paper, individual titres were reported for 5 subjects at both 14 and 28 days, the latter values were equivalent or only slightly lower than those measured at the earlier timepoint.

Table 5.7: Age dependent immunogenicity of Hib conjugate vaccines

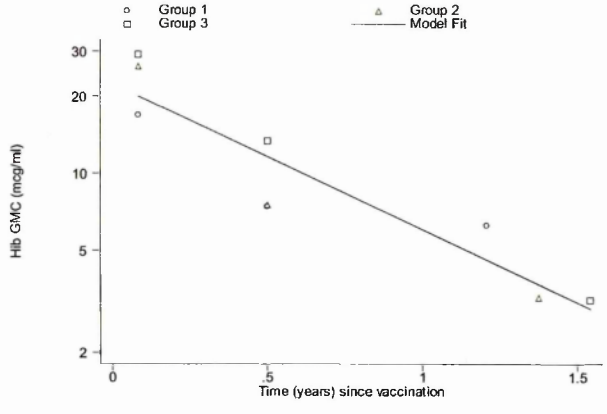
Study	Vaccine and Schedule	Age at Immunisation	Time From Immunisation	Hib GMC µg/ml
Heath PT 2000	Separate PRP-T	2,3,4 months	1 month	4.60
Goldblatt 1998	DTwP-PRP-T	2,3,4 months	1 month	6.23
Rothstein 1991	HbOC	7-11 months	2 months	3.84
Rothstein 1991	HbOC	12-15 months	2 months	8.31
Madore 1990b	HbOC	15-23 months	1 month	13.77
Makela 1987	PRP-D	32 years	14 days	44.54

A difficulty in estimating the anticipated response to immunisation from these data is the fact that antibody titres are reported 2 months following immunisation in 7-15 month olds. In order to estimate what they might have been one month after the vaccine, we must calculate the anticipated rate of antibody decline following conjugate vaccination and work backwards. The following data are from Madore's study of infants given three doses of conjugate vaccine, starting at 1-2 months, 3-4 months, and 5-6 months, respectively. They were then followed until 24 months of age and serum measured at intervals as follows (Madore D et al 1990a):

Table 5.8: Hib Antibody persistence following conjugate immunisation

Study Group	No	Average age at serum collection	Average time since primary course	Group 1 Hib GMC (µg/ml)	Group 2 Hib GMC (µg/ml)	Group 3 Hib GMC (µg/ml)
1	163	6.5 mos	1 month	16.84		
2	193	8.5 mos	1 month		26.23	
3	49	10.5 mos	1 month			29.11
1	88	11.5 mos	6 months	7.41		
2	188	13.5 mos	6 months		7.53	
3	46	15.5 mos	6 months			13.37
1	65	24 mos	14.5 months	3.2		
2	131	24 mos	16.5 months		3.28	
3	37	24 mos	18.5 months			6.25

Figure 5.7: Antibody titres by time from immunisation, with regression line

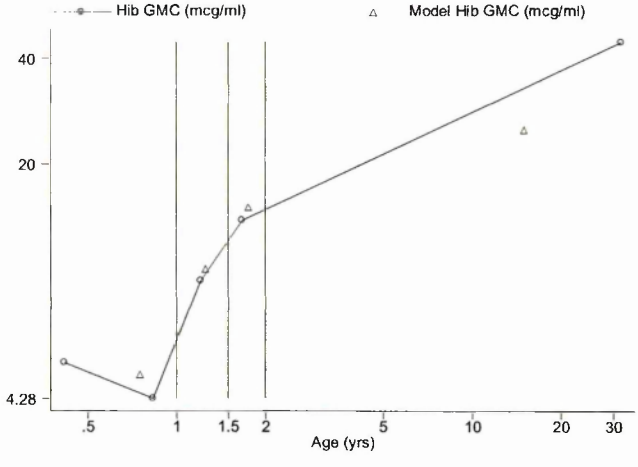


The equation describing the line of best fit for the regression model is:

ln (Hib GMC) = ln (post vaccination Hib GMC) – 1.3 × time (yrs) since vaccination

Taking this rate of decline into account, antibody titres 1 month following immunization at 7-11 months, and 12-15 months, would be anticipated to have been 4.28 and 9.26 μg/ml, respectively. Figure 5.8 graphs the observed data on antibody response to conjugate vaccines by age, with superimposed estimates of the peak concentration following exposure to be used in the model for the four age groups defined. In contrast to the slower development of responsiveness to polysaccharides, observed titres following administration of conjugate vaccine rise rapidly over the first two years of life. For this reason, divisions between age classes used to reflect maturation of the immune response differ from those previously used: 0.5-0.99 yrs (5.0 μg/ml), 1-1.49 yrs (10.0 μg/ml), 1.5-1.99 yrs (15.0 μg/ml) and 2 years and over (25.0 μg/ml). Vertical bars define the age categories distinguished in the model.

Figure 5.8: Post conjugate immunisation Hib antibody GMC by age



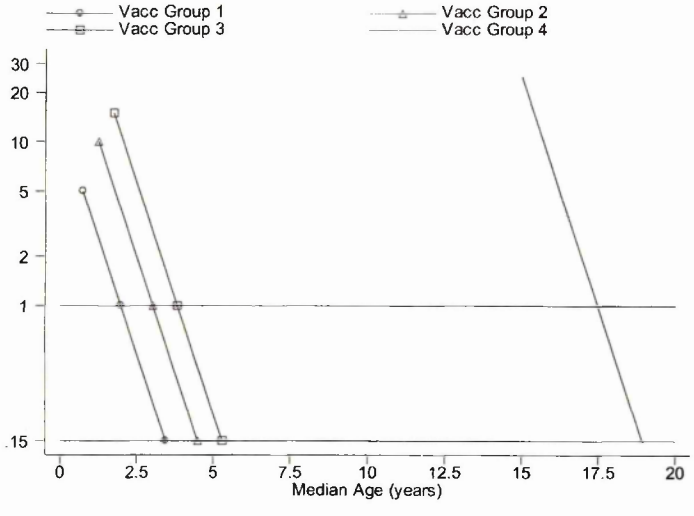
Parameter estimation

Once again, in order to determine the duration of high antibody protection following immunization at different ages, information on the peak response achieved is combined with knowledge of the post conjugate immunization waning rate in order to derive the time taken to achieve the threshold titre of 1.0 μg/ml. The derived parameter values are shown in Table 5.9 and Figure 5.9.

Table 5.9: Age dependent waning rates following Hib conjugate vaccination

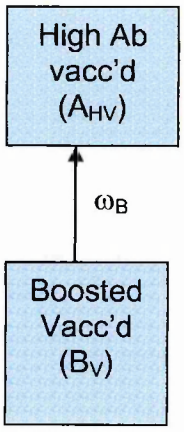
Age	Post Exposure Hib GMC μg/ml	Time to wane to 1.0 μg/ml	Parameter estimated	Waning rate
0.5–1 years	5.0	1.23 years	ω_{HV1}	0.81
1-1.5 years	10.0	1.77 years	ω_{HV2}	0.56
1.5-2 years	15.0	2.08 years	ω_{HV3}	0.48
2-20 years	25.0	2.48 years	ω_{HV4}	0.40

Figure 5.9: Age dependent waning following Hib conjugate immunisation



Calculating the rate of decline from 1.0 to 0.15 μg/ml necessitates only one further calculation, resulting in an estimated time to waning of 1.46 years, with a parameter value for ω_{LV} of 0.69.

5.3.4 Duration of immunity following re-exposure to Hib in vaccinated individuals (ω_B)



Literature Review

Response to a booster dose of conjugate vaccine has been assessed at 12 months of age following DTwP-PRP-T given according to the British accelerated schedule (Goldblatt D et al 1998d). In this study, the Hib GMC was 108.39 μg/ml (95% CI 91.62, 128.23). In Canada, the boost response to PRP-T in children 4-5 years of age primed with the same vaccine at 2, 4, 6 and 18 months was assessed, and the GMC was found to be 102 μg/ml (79.9, 129) (Scheifele DW et al 1999). Ten years following receipt of four doses of PRP-D between 3 and 18 months, a cohort of Finnish children showed persistent memory responses to a dose of PRP, achieving a GMC of 107.7 μg/ml (88.2, 131.4) compared with 28.6 (23.3, 35.0) in those not primed in infancy (Makela PH et al 2003).

Justification for using these estimates as a surrogate of natural exposure comes from Barbour's observation of higher antibody titres in association with Hib carriage in pre-school aged children vaccinated in infancy, compared with those who had not been vaccinated (Barbour ML et al 1993). Five children who had been vaccinated in infancy were identified as carriers – their median Hib antibody concentration was 65.6 μg/ml (interquartile range: 27.1, 102.8). In contrast, the three carriers who had not been previously immunised had a median titre of 3.51 μg/ml (0.2, 31.2).

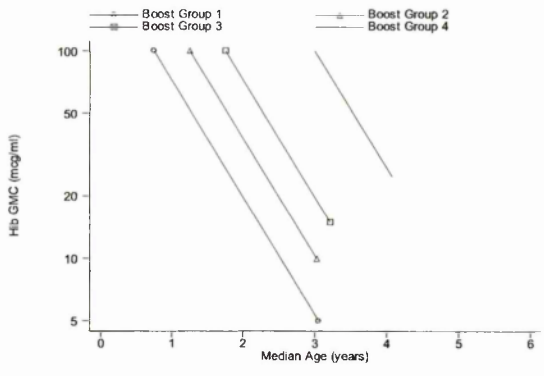
Parameter Estimation

In order to calculate the time spent in the 'boosted' immunity category (B_V) several pieces of data must be combined. Let us assume that the peak boost response is 100 μg/ml, and is independent of age. The rate of decline of antibody in conjugate vaccine immunised individuals has already been calculated above. In addition, peak post immunisation antibody titres have been assigned to each of the four age classes. Time in the boosted category is therefore age dependent, being the time taken to wane from 100 μg/ml to 5, 10, 15 or 25 μg/ml, depending upon the age at boosting. Final parameter values based on these data are shown in Table 5.10 and represented in Figure 5.10.

Table 5.10: Age dependent waning rates following boosting of Hib immunity through natural exposure – time to wane into high antibody vaccinated (A_{HV}) category

Age	Post Primary Hib GMC μg/ml at this age	Time to wane from 100.0 μg/ml	Parameter estimated	Waning rate
0.5–1 years	5.0	2.30 years	ω_{B1}	0.43
1-1.5 years	10.0	1.77 years	ω_{B2}	0.56
1.5-2 years	15.0	1.46 years	ω_{B3}	0.69
2-20 years	25.0	1.07 years	ω_{B4}	0.94

Figure 5.10: Age dependent waning following re-exposure to Hib in vaccinated children



5.4 Relative protection against acquisition from low antibody state, θ

Studies relating Hib antibody titres to protection against acquisition of colonisation are described in Section 5.3 a) above. In the initial parameterisation of the model, it was assumed that no protection against carriage was provided by serum antibody concentrations <1.0 μg/ml. For this reason, protection against infection in the low antibody state (θ) was set to 0. It was incorporated within the model in order to allow later sensitivity analysis to test this assumption.

5.5 Force of infection, λ(a)

The age dependent force of infection was calculated using the same ordinary differential equation model used to estimate duration of natural immunity, in section 5.3 a). Calculated estimates of these parameter values are shown in Table 5.11.

Table 5.11: Parameter estimates for the age dependent force of infection

Age	Parameter Estimated	Mean Value
0.5-1.99 years	λ_1	0.45
2-4.99 years	λ_2	0.22
5-10.99 years	λ_3	0.40
11-20.5 years	λ_4	0.55

5.6 Calculation of invasive disease incidence

Literature Review

While the model predicts that 60% of British children would have been exposed to Hib by the age of 5 years (Figure 5.6), only 30 per 100,000 children in this age group experienced invasive Hib infection every year before vaccine introduction (Anderson EC et al 1995). As such, invasive disease episodes made only a very small contribution to the total force of infection in the population. Further, due to the ready availability of effective therapy, the case fatality rate for Hib infections in Britain was less than 5% prior to vaccine introduction (Anderson EC et al 1995) and has remained stable (Heath PT et al 2000b) meaning that this infection has a negligible impact on total population size. For these reasons, invasive disease has been calculated as a quasi-steady state approximation of the number of colonised individuals in the population at any point in time.

The instantaneous incidence of Hib disease was determined by the balance between risk of progression to bacterial invasion, and rate of recovery from the illness. With an average antibiotic treatment course lasting five days, the duration of invasive disease was estimated at one week. The risk of developing disease during each episode of carriage is more complicated to ascertain. Figure 1.2 shows the exquisitely age dependent distribution of Hib disease incidence, with the vast majority of episodes occurring within the first 12 months of life when the prevalence of colonisation is relatively rare, declining rapidly thereafter.

At a population level, these trends have been explained in terms of the prevalence of antibody to the Hib capsular polysaccharide PRP. Maternal antibody provides some passive protection against infections for the first few months of life. After this is lost, Hib incidence rises, with falling rates from the second year of life onwards correlating with the development of natural immunity through exposure to Hib or cross reactive organisms (Fothergill LD et al 1933) (Anderson P et al 1977a) (Kayhty H et al 1983a) (Figure 1.3 a), b), c)). A rhesus monkey model of invasive Hib infection has confirmed the increased susceptibility of very young animals to meningitis following established bacteraemia, due to reduced efficiency of reticuloendothelial clearance of organisms in the bloodstream (Smith AL 1987). Monkeys older than 2 months of age are able to contain bacteraemia so that equilibrium is maintained over several days. Those less than two months are vulnerable to rapid invasion of the cerebrospinal fluid, due to uncontrolled bacterial growth in the blood.

The age cohort model provided a framework within which to consider questions regarding this age dependent susceptibility to infection:

a. Can the age distribution of infections be solely explained by the higher prevalence of measurable Hib antibody, providing protection against bacterial invasion, in older age groups?

b. What is the protective efficacy of immunologic memory once antibody wanes? Should a history of past exposure alone prove insufficient to explain the age distribution of infection:

c. Are there factors other than the absence of antibody which place young infants at additional risk of serious bacterial infection, thus requiring an age dependent parameter to describe progression to invasive disease?

Parameter Estimation

The quasi steady state approximations were defined according to the following series of equations, which describe the risk of invasive disease for each of the infected states described in the model. The average duration of infection (1/ν) is one week, so the rate of recovery per year $\nu = 52$. Progression to invasive disease occurs at a rate δ, which is here denoted as age dependent, a feature to be explored in the sensitivity analysis. The efficacy of detectable antibody at preventing progression to bacterial invasion at the point of exposure to Hib is designated θ, and protection due to memory after antibody has waned is denoted μ, where $0 \leq \mu < \theta \leq 1$. No additional protection of antibody induced by vaccination as compared with natural exposure was assumed.

$$\frac{dD_F}{dt} = \delta(a)F(a,t) - \sigma D_F(a,t) \qquad D_F(a,t) = \frac{\delta(a)F(a,t)}{\sigma}$$

$$\frac{dD_{IL}}{dt} = (1-\theta)\delta(a)I_L(a,t) - \sigma D_{IL}(a,t) \qquad D_{IL}(a,t) = \frac{(1-\theta)\delta(a)I_L(a,t)}{\sigma}$$

$$\frac{dD_{IN}}{dt} = (1-\mu)\delta(a)I_N(a,t) - \sigma D_{IN}(a,t) \qquad D_{IN}(a,t) = \frac{(1-\mu)\delta(a)I_N(a,t)}{\sigma}$$

$$\frac{dD_{ILv}}{dt} = (1-\theta)\delta(a)I_{LV}(a,t) - \sigma D_{ILv} \qquad D_{ILv}(a,t) = \frac{(1-\theta)\times\delta(a)\times I_{LV}(a,t)}{\sigma}$$

$$\frac{dD_{INv}}{dt} = (1-\mu)\delta(a)I_{NV}(a,t) - \sigma D_{INv} \qquad D_{INv}(a,t) = \frac{(1-\mu)\delta(a)I_{NV}(a,t)}{\sigma}$$

The case per carrier ratio was calculated in Excel, based on weekly model outputs of infection prevalence, by age and type, prior to introduction of vaccination. This was compared against the observed pre vaccine case incidence in England and Wales by age from the combined HPA HRU/CDSC dataset. Deviance between these two sets of values was minimized using a log-likelihood method by simultaneously solving for optimal values of $\delta(a)$, θ and μ. Parameter values were initially calculated using the base case assumption that the protection afforded by low levels of antibody against acquisition (ε) was equal to 0. A result of this critical assumption was that the model could not be solved for values of θ and μ which were greater than 0. Estimates for the age specific progression to invasive disease, $\delta(a)$, are shown in Table 5.12.

Table 5.12: Age dependent rates of progression to invasive disease, $\delta(a)$

Age	Parameter Estimated	Average Rate of Progression to Invasive Disease
0.5-0.99 years	δ_1	0.0127
1-1.99 years	δ_2	0.0042
2-2.99 years	δ_3	0.0037
3-3.99 years	δ_4	0.0030
4-4.99 years	δ_5	0.0016
5-20.5 years	δ_6	0.00006

5.7 Transmission coefficient, β

Initial conditions for the model were calculated using the age dependent forces of infection derived above. The force of infection was further defined as follows, where *C(a,a')* determines the influence of infectious people of age class *a'* in contributing to new infections in age class *a* and *β(a)* denotes the age dependent susceptibility to acquisition of individuals in age class *a*.

$$\lambda(a,t) = \int_{0.5}^{20.5} C(a,a')\,\beta(a)[F(a',t)da' + I_L(a',t)da' + I_N(a',t)da' + I_{LV}(a',t)da' + I_{NV}(a',t)da']$$

This may be simplified to

$$\lambda(a,t) = \int_{0.5}^{20.5} C(a,a')\,\beta(a)\,[Y(a',t)da']$$

where $Y(a',t)da' = [F(a',t)da' + I_L(a',t)da' + I_N(a',t)da' + I_{LV}(a',t)da' + I_{NV}(a',t)da']$

This effective contact parameter $C\beta$ is a two dimensional step function that can be represented by a matrix of constants, shown below. Values of *β(a)* were calculated according to the method of Anderson and May (Anderson RM et al 1985) using the equilibrium distribution of the number of infections by age, to comprise the elements of a 'Who Acquires Infection From Whom' (WAIFW) matrix under a number of different assumptions of the relative values of *C(a,a')*. The WAIFW matrix was then used to calculate the force of infection λ at all subsequent time steps based on the number of carriers by age at each preceding time step.

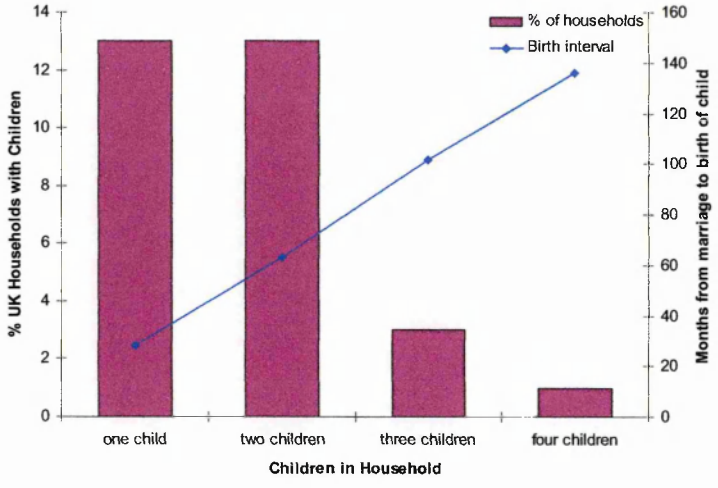
$$\begin{bmatrix}\lambda_1\\ \lambda_2\\ \lambda_3\\ \lambda_4\end{bmatrix}=\begin{bmatrix} C_{11}\beta_1 & C_{12}\beta_1 & C_{13}\beta_1 & C_{14}\beta_1\\ C_{21}\beta_2 & C_{22}\beta_2 & C_{23}\beta_2 & C_{24}\beta_2\\ C_{31}\beta_3 & C_{32}\beta_3 & C_{33}\beta_3 & C_{34}\beta_3\\ C_{41}\beta_4 & C_{42}\beta_4 & C_{43}\beta_4 & C_{44}\beta_4\end{bmatrix}\times\begin{bmatrix}Y_1\\ Y_2\\ Y_3\\ Y_4\end{bmatrix}$$

The rate at which individuals of one age class transmit infections to others cannot be directly observed in populations. Components of social behaviour between potential hosts which contribute to this likelihood can, however, be used to infer a range of plausible contact matrices based on pre-vaccination data. These may be subjected to a sensitivity analysis. The contact matrix may thus be described:

$$\begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14}\\ C_{21} & C_{22} & C_{23} & C_{24}\\ C_{31} & C_{32} & C_{33} & C_{34}\\ C_{41} & C_{42} & C_{43} & C_{44}\end{bmatrix}$$

For infants and young children, the likelihood of contact with an older sibling in the home environment can be inferred from national data collected on household size and birth intervals (www.statistics.gov.uk/statbase a, b) (Figure 5.11). These data show that just over half of all children in households have contact with an older sibling who is on average three years older. Approximately one in eight will be exposed to a further sibling, an average of six years older. Prior to the use of widespread vaccination, the presence of an older sibling in the household was a recognised risk factor for invasive Hib infection, suggesting that the older child acted as a source host (Ounsted C 1950, 1951) (Cochi SL et al 1986).

Figure 5.11: Household size and birth interval



Patterns of mixing outside the home have been changing over the past ten years. The number of children under the age of eight cared for by nurseries and professional childminders in England, Wales and Northern Ireland has risen from 400,000 in 1992 to 631,000 in 1999 – the equivalent of an annual birth cohort for the region (www.statistics.gov.uk/statbase c). The social exuberance of toddlers, combined with their liberal sharing of nasal secretions, particularly facilitates transmission of nasopharyngeal commensals in day care (Murphy TV et al 1985). Increased relative risk of Hib disease in unvaccinated American day care attendees in the 1980s ranged from 12.3 (95% CI 4.8, 31.1) for children less than 1 year of age to 3.8 (0.9, 15.7) in 2-3 year olds (Redmond SR et al 1984). Over the age of five, mixing with large numbers of peers within age classes occurs in a structured and more consistent way due to compulsory school education, although contact is not likely to be as close. The framework illustrated in Figure 5.12 was used for considerations of the likely proportionate contribution of mixing between different age groups to acquisition of infections:

Figure 5.12: Mixing assumptions to be explored in the contact matrix

Age Group	0-2 yrs	2-5 yrs	5-11 yrs	11-20 yrs
0-2 yrs	Limited unless Nursery	Close Sibling Mixing	Limited Sibling Mixing	Negligible Contact
2-5 yrs	Close Sibling Mixing	Nursery Play Group	Moderate Sibling Mixing	Negligible Contact
5-11 yrs	Limited Sibling Mixing	Moderate Sibling Mixing	Primary School	Distant Sibling Mixing
11-20 yrs	Negligible Contact	Negligible Contact	Distant Sibling Mixing	Secondary School University

Under an assumption of uniform mixing in the population, the contact matrix would look as follows:

$$\begin{bmatrix} 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 \end{bmatrix}$$

The most extreme assortative mixing assumption would be:

$$\begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

A range of matrices were explored in the sensitivity analysis, with a typical configuration based on the assumptions in the table above being:

$$\begin{bmatrix} 1 & 100 & 1 & 0 \\ 100 & 100 & 10 & 0 \\ 1 & 10 & 100 & 1 \\ 0 & 0 & 1 & 100 \end{bmatrix}$$

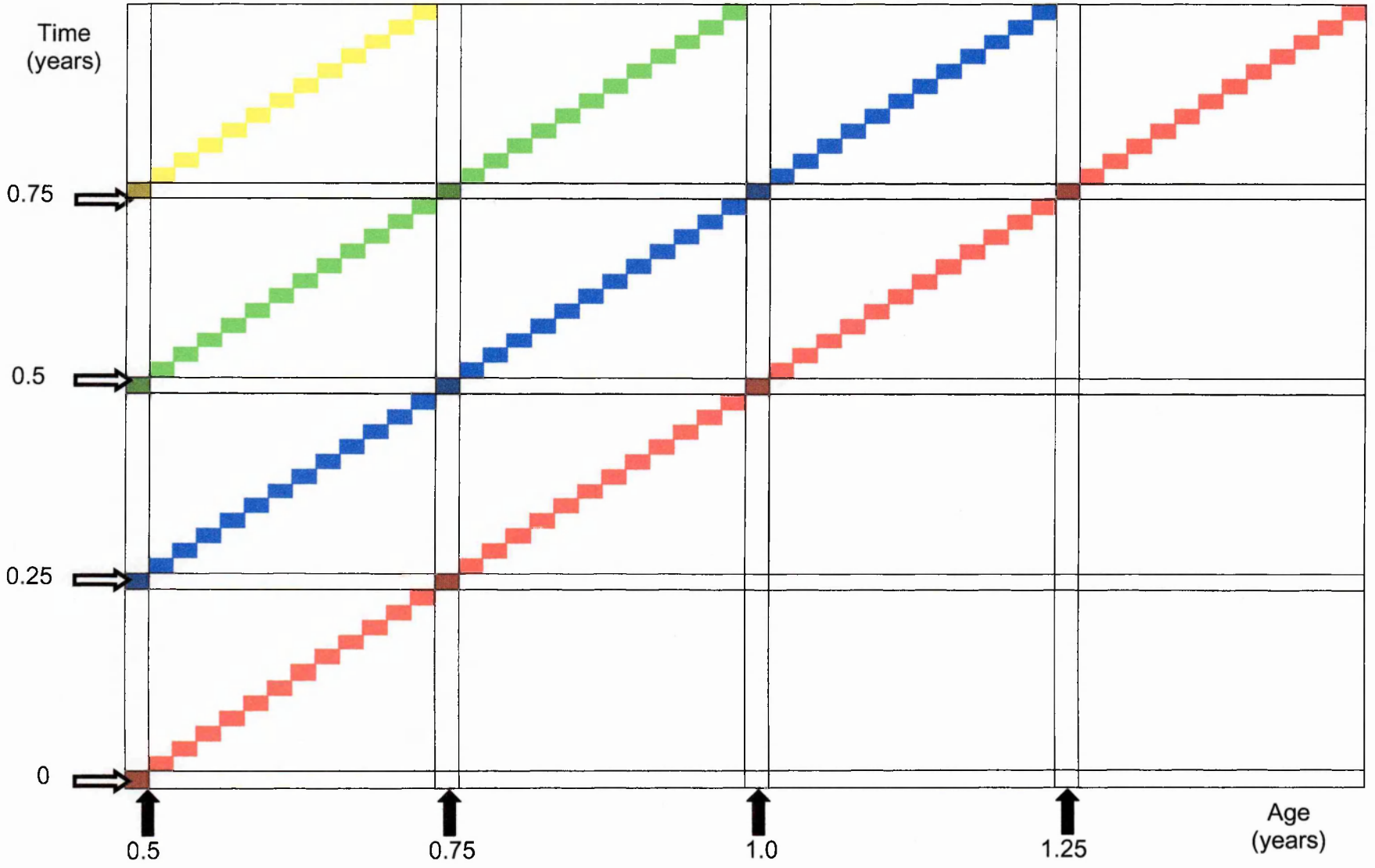
5.8 Numerical Techniques

The method of characteristics was used to find numerical solutions to the partial differential equations (PDEs) described in Section 4.1. These were reduced to a series of ordinary differential equations (ODEs) along the characteristic lines $t = a +$ *constant,* within eighty sequential three-month age cohorts. The equations were solved by fourth order Runge-Kutta integration using Model Maker Version 4 software (Cherwell Scientific, UK) with a step length of one week. The time step was chosen to reflect the speed with which transitions were occurring in the model. This provided suitable precision as no change in either the dynamics or the distribution of individuals in the population at equilibrium was noted with further reduction of the time interval. As the PDEs were a series of integro-differential equations, the force of infection for each age category was integrated and updated at each time step.

A schematic representation of the model over one year is shown in Figure 5.13, illustrating births into the model every three months. As aging occurred weekly, there were potentially thirteen birth cohorts in any given three month time period, but the figure illustrates the progression of just one such birth cohort. Outputs were recorded every three months, so that each cohort was viewed at only one age for comparison over time. Cohorts exited from the model and were lost to consideration after the age of 20.5 years.

The benefit of this method was the ability to use an established software package to perform the numerical integrations, without the need to write new code. The software was, however, quite cumbersome, and the package rather inflexible, making any changes to the model time consuming.

Figure 5.13: Schematic Representation of the Age Cohort Model (⇧ births into model; ⬆ aging to next cohort; ▨ model output)



5.9 Code Testing

Numerical accuracy of the age structured model was tested at a number of stages of development by different means.

Aging

In the first instance, the simulation of the unvaccinated population was tested by introducing a single naïve birth cohort at time 0, and charting its progress through the model compartments over time until age 20.5 years. All age groups were summed across compartments at each time point in order to ensure consistency of population size. Outputs were visually checked to ensure that the cohort moved on to the next age category at the appropriate time. Secondly, the model was run with a series of birth cohorts introduced three monthly with p, q and z set at 0, until the total population size achieved was 500,000 and all compartments were at equilibrium. The distribution of the population by age at a single time point at equilibrium was compared with that observed in the Excel spreadsheet model described earlier, which was used to parameterise the force of infection.

Calculation of transmission coefficient, β

The number of infections by age group at equilibrium before introduction of vaccination was derived by running the model using the values calculated for the age specific force of infection, $\lambda(a)$. Numerical values for the effective transmission coefficients, $C_{ij}\beta_i$ were then calculated using the matrices described in section 7 above. Initial values of all compartments for models using β were imported from the equilibrium distribution observed in the preceding λ model for that given parameter set. Each β model was allowed to run for a period of at least five years before

immunisation was imposed. In this way, it was possible to check that the number of infected individuals remained constant over this period (ie λ remained constant) and that no numerical error had been introduced in the analytic calculation of β.

Introduction of vaccination

In order to ensure that no errors had been introduced in the model describing the vaccinated population, each new version of the model was checked in an Excel spreadsheet. Outputs from all 13 compartments were summed within each age category at each time point. These were visually checked to ensure that all were equal to the birth cohort size of 6,250. They were further summed across age categories for each time point in order to ensure that the total population size remained 500,000.

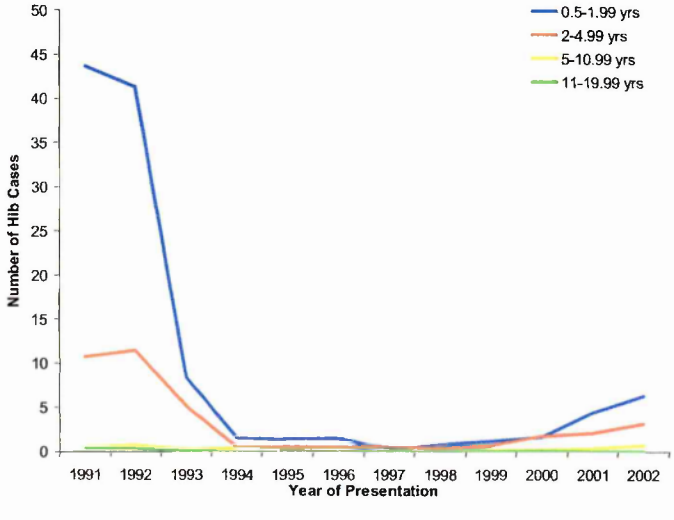
Chapter 6: Predicted Time Series Under Different Model Assumptions

6.0 Introduction

The model was designed to allow exploration of key theories about vaccine protection and subsequent effects on Hib transmission and immunity in the population. For this reason, we were more interested in investigating the structural sensitivity of the model than in conducting a formal sensitivity analysis using randomly sampled parameter values, as in risk analysis models (Vose D 1996).

Our aim was to recapitulate the observed time series for all four population measures for which UK data was available: prevalence of high and low serum antibody levels, carriage prevalence and invasive disease incidence. In exploring the behaviour of the system under vaccination, the most difficult thing to achieve was a late recurrence of disease in children under five years of age following an initial period of effective control, shown in the population data represented in Figure 6.0.

Figure 6.0: Invasive Hib disease reports by age group, England and Wales 1991-2002



6.1 Effects of vaccine scheduling and immunogenicity

A number of aspects of Hib vaccine introduction and use in the UK have been unique in comparison with other developed countries. In order to understand the reasons why the UK has observed an increase in disease not experienced in those countries, the effects of the initial catch-up immunisation campaign and the recent switch to less immunogenic DTaP-Hib combination vaccines needed to be explored. The model was run using the parameter set summarised in Table 5.0 of Chapter 5 and immunisation was introduced at time t=5 years, corresponding to the calendar year 1993 when comparing outputs with observed data. An initial assumption was made of uniform mixing between all age classes (Section 5.7). Additional complexities were added during sequential simulations as follows:

a) Introduction of immunisation without a catch-up campaign

b) Introduction of immunisation with a catch-up campaign, staggered at quarterly intervals over the first year of vaccine introduction, using the coverage figures from the South East Thames region shown in Table 5.3 of Chapter 5.

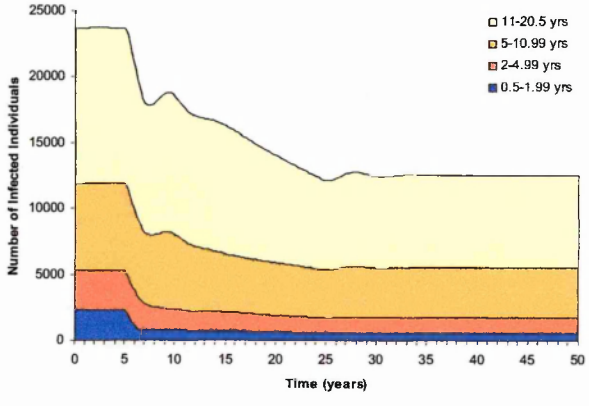
c) Introduction of immunisation with a catch-up campaign as in part b), and with the additional influence of use of a poorly immunogenic vaccine in years 12 and 13 (corresponding to 2000/2001), administered to one third of the immunised population. Values calculated for p, q and z during these years are shown in section 5.1.1 of Chapter 5.

Figure 6.1 shows the total number of infections in each age class under each of these three conditions. It can be seen that catch-up immunisation produces a more precipitous fall in the number of infections immediately following vaccine introduction, but a large rebound occurs

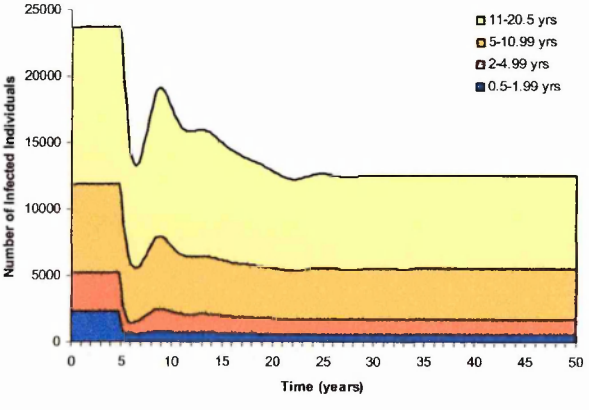
predominantly within the older age groups in only a few years. The use of a poorly immunogenic vaccine seven years later results in a second peak of infections which affects all age classes. Both of these factors, then, may be expected to have a profound effect on the epidemiology of Hib in the UK population, justifying the necessity of their inclusion in the model.

Figure 6.1: Effects of vaccine scheduling and immunogenicity on total number of infections in the population, by age class and time (Vaccine introduction: t=5 years)

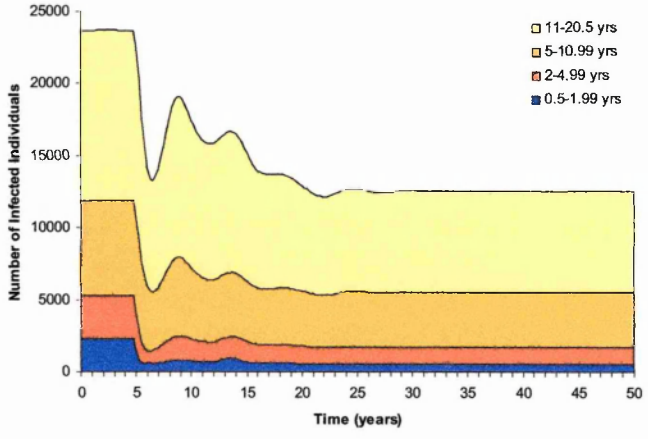
a) Without catch-up immunisation



b) With catch-up immunisation



c) With catch-up immunisation and use of poorly immunogenic vaccine in years t=12 and t=13 (corresponding to 2000/2001)



In attempting to understand how this bimodal peak in total infections may relate to observed trends in Hib disease in the UK, one must bear in mind changes in the proportion of children under five years of age with protective antibody levels between 1994 and 2000 (Section 2.6.2) (Trotter CL et al 2003b). Three years following the introduction of immunization, toddlers demonstrated very high levels of specific antibody against Hib as a result of the catch-up immunization campaign. We postulate that this immunity was sufficient to protect them against disease, even in the face of an increase in circulation of the organism among their older siblings five years after vaccine introduction. A second rise in infections coincided with a period when poorly immunogenic vaccines were being used, and was probably additionally fuelled by the increase in the size of the pool of susceptible infants. As the risk of bacterial invasion is exquisitely dependent on both age and antibody status at the point of exposure to the pathogen (Section 5.6 of Chapter 5), any reduction in immunity in the population under 5 years may be expected to have a marked effect on the number of Hib disease cases for a given background level of carriage.

6.2 Esimation of the interepidemic interval of the SIRS system

An alternative explanation would be that the dynamics of the model as configured are incorrect, and that the first peak of infections appears too early. A simple SIRS ordinary differential equation model was developed in order to explore this possibility further, by enabling analytic calculation of the anticipated interepidemic interval following perturbations of the system. It is described by the following series of equations where S, I and R refer to the susceptible, infected and resistant states respectively and total population size N=S+I+R.

$$\frac{dS}{dt} = (1\text{-}p)B - (\lambda+\mu)S + \omega R$$

$$\frac{dI}{dt} = \lambda S - (\upsilon+\mu)I$$

$$\frac{dR}{dt} = \upsilon I - (\omega+\mu)R$$

In this simple model, births (B) balance deaths (μN) resulting in a constant population size (N) of 500,000. Initial conditions are defined for an unvaccinated population where the proportion vaccinated, p=0. All individuals under this condition are born into a susceptible class (S) from which they acquire infections at a rate dependent on the force of infection, λ. The average duration of infection is equivalent to the reciprocal of the recovery rate, $1/\upsilon$. Following recovery, a period of resistance to reinfection (R) results, with immunity waning at a rate ω to return individuals to the fully susceptible state. The duration of protective immunity was an average of $1/\omega$ across all four age classes in the age structured model, calculated in Section 5.3. This model described above was run to equilibrium and a value for the transmission coefficient, β, calculated where $\lambda = \beta I$. The force of infection was integrated and updated at each time step. The initial parameter set used in the model is shown in Table 6.0.

Table 6.0: Base Case Parameters in the SIRS Model

Model Parameter	Mean Value	Mean Duration in Class
Birth rate, B	10,000 per year	
Death rate, μ	0.02 per year	50 years
Force of infection, λ	0.4	
Rate of recovery from infection, υ	5	2.4 months
Rate of waning of immunity, ω	0.5	2 years

At equilibrium using the baseline parameter set, the proportion of the population that was infected was 4.3%, and the proportion resistant 41.5%. Both of these values were compatible with the UK data from 1990/1991 on which the age structured model was parameterized. The model was then run using a range of values for λ and ω in order to study the effects of changing these parameters on the equilibrium distribution of individuals in the population, and to allow calculation of β. The duration of carriage, υ, was fixed at 2.4 months. The system was perturbed by moving 20% of the susceptible class to the resistant class at a single time point, and the behaviour of the system observed under each of these conditions. A series of perturbations from 10% to 40% were explored, with similar outcomes.

Numerical outputs of the models and perturbations are represented below. Figure 6.2 shows changes in the proportion of the population who are resistant, for a range of values of λ and ω. The horizontal line indicates the observed value of approximately 40%. Figure 6.3 shows the corresponding prevalence of infections, in relation to a line denoting the observed 4%. The time to first rebound of infections, following the initial decline induced by a 20% perturbation, is shown under the same assumptions (Figure 6.4).

Figure 6.2: Proportion of the population who are resistant

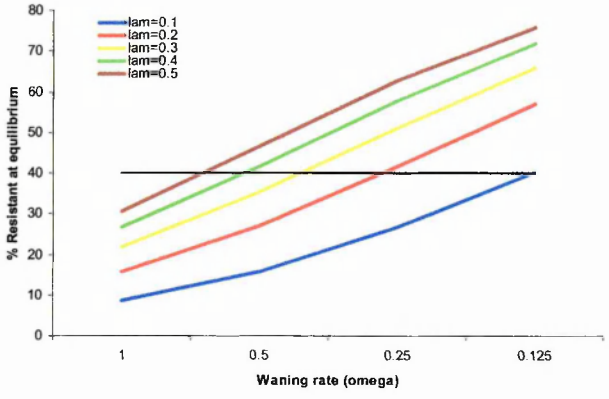


Figure 6.3: Proportion of the population who are infected

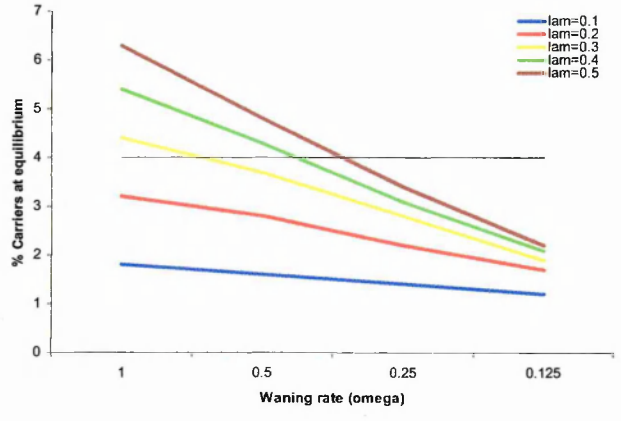
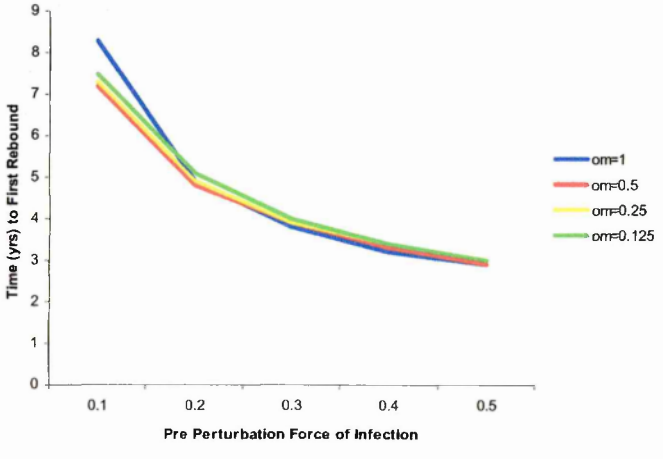


Figure 6.4: Time to first rebound of infections following 20% perturbation



As can be seen from Figure 6.4, the critical parameter here determining the time to rebound of infections was the pre-perturbation force of infection. For a force of infection of 0.2 or greater, the interepidemic interval was five years or less. Even for the lowest force of infection studied (λ=0.1) the longest time period to initial peak rebound of infections was 8 years, given the fixed assumption of duration of carriage of 2.4 months. From Figures 6.2 and 6.3, it can be seen that a force of infection this low would be inconsistent with the observed distribution of resistant and infected individuals in the population at baseline. Analytic solutions were sought to describe the properties of this system further.

6.2.1 The damped harmonic oscillator

If we assume that the death rate, μ, is 0 until a given age and infinite thereafter, the model can be simplified as follows to exclude consideration of births and deaths:

$$\dot{S} = -\lambda S + \omega R \qquad (1)$$

$$\dot{I} = \lambda S - \upsilon I \qquad (2)$$

$$\dot{R} = \upsilon I - \omega R \qquad (3)$$

This series of three equations was then reduced to two, based on the knowledge that total population size, N = S + I + R, therefore:

$$I = N - S - R \qquad (4)$$

(4) into (3): $\dot{R} = \upsilon N - \upsilon S - \upsilon R - \omega R$

$$\dot{R} = K - \upsilon S - (\upsilon + \omega) R \qquad (5) \quad \text{where } K = \upsilon N$$

Based on equations (1) and (5), the system could be described in the form of a second order linear ODE, derived as follows. From equation (1):

$$\omega R = \dot{S} + \lambda S$$

$$R = \frac{1}{\omega} (\dot{S} + \lambda S) \qquad (6)$$

(6) into (5): $$\dot{R} = K - \upsilon S - \frac{1}{\omega} (\upsilon + \omega) (\dot{S} + \lambda S) \qquad (7)$$

Also from equation (1):

$$S = \frac{\omega}{\lambda} R - \frac{1}{\lambda} \dot{S}$$

Differentiating this equation, we obtain:

$$\dot{S} = \frac{\omega}{\lambda} \dot{R} - \frac{1}{\lambda} \ddot{S} \qquad (8)$$

Rearranging this:

$$\dot{R} = \frac{\lambda}{\omega} \dot{S} + \frac{1}{\omega} \ddot{S} \qquad (9)$$

Thus RHS (7) = RHS (9):

$$\frac{\lambda}{\omega}\dot{S} + \frac{1}{\omega}\ddot{S} = K - \upsilon S - \frac{1}{\omega}(\upsilon + \omega)(\dot{S} + \lambda S)$$

$$\ddot{S} + (\lambda+\nu+\omega)\dot{S} + (\lambda\nu+\lambda\omega+\omega\nu)S - \omega K = 0 \qquad (10)$$

This second order linear ODE takes the general form (Jordan DW et al 1999):

$$\ddot{x} + k\dot{x} + cx = 0,$$

The discriminant of this equation, $\Delta = k^2 - 4c$, describes the oscillatory behaviour of the system. If $\Delta>0$ (overdamped) or $\Delta=0$ (critically damped), the magnitude of oscillations will decrease exponentially. If $\Delta<0$, the system is described as a damped oscillator. For negative values of Δ, the oscillatory frequency can be estimated as follows:

$$\text{Oscillatory frequency} = \frac{(-\Delta)^{1/2}}{4\pi}$$

Δ was calculated, given estimates of λ ranging from 0.1 to 0.5 and of ω ranging from 0.05 to 2.5. The duration of infection remained fixed at 2.4 months. All values for the discriminant within the parameter space explored for the model above were >0. As such, the system was characterised as overdamped, enabling us to predict that transient behaviour induced by perturbations would settle relatively quickly to a new stable equilibrium without an extended period of oscillations. While of use in describing the characteristics of the system, this method did not allow us to derive an analytic solution for the interepidemic period.

6.2.2 Analytic solution for the interepidemic interval

Analytic results for the dynamics of epidemic and endemic oscillations in models of infection and immunity are discussed in Appendix C of Anderson and May's textbook (Anderson RM et al 1990). In particular, the properties of models in which immunity is not life-long are considered. The resulting 'steady input of fresh infectives...undercuts the propensity to oscillate', as has been seen from the demonstration of overdamping in Section 6.2.1 above. Their approach is taken to our original equations for the SIRS system:

$$\frac{dS}{dt} = (1\text{-}p)B - (\lambda+\mu)S + \omega R \qquad (1)$$

$$\frac{dI}{dt} = \lambda S - (\upsilon+\mu)I \qquad (2)$$

$$\frac{dR}{dt} = \upsilon I - (\omega+\mu)R \qquad (3)$$

Where $R = N - S - I$ (4)

(4) into (1): $$\frac{dS}{dt} = \mu N - (\lambda+\mu)S + \omega(N\text{-}S\text{-}I) \qquad (5)$$

where: $$I = \frac{\lambda}{\beta} \qquad (6)$$

(6) into (5): $$\frac{dS}{dt} = (\mu+\omega)N - (\lambda+\mu+\omega)S - \frac{\omega\lambda}{\beta}$$

If we consider the proportion susceptible, s:

$$\frac{ds}{dt} = (\mu + \omega) - (\lambda + \mu + \omega)s - \frac{\omega\lambda}{\beta N} \qquad (7)$$

Where: $$R_0 = \frac{\beta N}{(\nu + \mu)} \qquad (8)$$

(8) into (7): $$\frac{ds}{dt} = \mu + \omega - \lambda s - \mu s - \omega s - \frac{\omega\lambda}{Ro(\nu + \mu)}$$

$$\frac{ds}{dt} = \mu - (\mu + \lambda)s + \omega\left(1 - s - \frac{\lambda}{Ro(\nu + \mu)}\right) \qquad (9)$$

Similarly,

$$\frac{dI}{dt} = \lambda S - (\upsilon + \mu)I \qquad (2)$$

(6) into (2): $$\frac{dI}{dt} = \beta IS - (\upsilon + \mu)I$$

$$\frac{dI}{dt} = I[\beta S - (\upsilon + \mu)] \qquad (10)$$

As I and λ differ only by the proportionality constant, β:

$$\frac{d\lambda}{dt} = \lambda[\beta S - (\upsilon + \mu)]$$

If we consider the proportion infected, i, in terms of λ:

$$\frac{1}{N}\frac{d\lambda}{dt} = \lambda[\beta s - \frac{(\nu+\mu)}{N}]$$

$$\frac{d\lambda}{dt} = \lambda\,(\nu+\mu)[\frac{\beta N s}{(\nu+\mu)} - 1] \qquad (11)$$

(8) into (11): $$\frac{d\lambda}{dt} = \lambda\,(\nu+\mu)[R_0 s - 1] \qquad (12)$$

Within this system, the endemic equilibrium is:

$$s^* = 1/R_0$$

$$\lambda^* = \frac{(\nu+\mu)(Ro-1)(\omega+\mu)}{(\omega+\nu+\mu)}$$

The general equation for the interepidemic interval is:

$$T \approx 2\pi(AD)^{1/2}$$

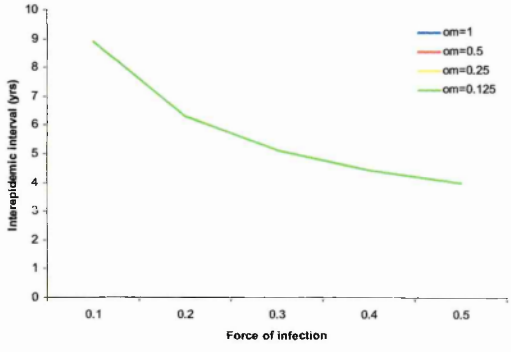
Where $$A = 1/\lambda^*$$

Thus, $$A = \frac{(\omega+\nu+\mu)}{(\nu+\mu)(Ro-1)(\omega+\mu)} \qquad (13)$$

And $$D = 1/\nu \qquad (14)$$

Values for T were calculated for the range of parameter values explored numerically above, with the following results:

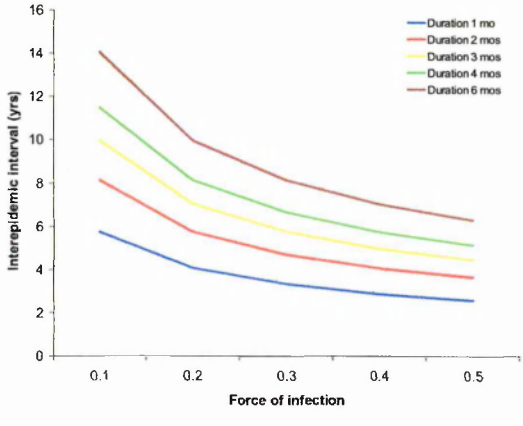
Figure 6.5: Analytic solution for the interepidemic period, for different values of λ and ω



These values can be seen to be very similar (although slightly larger) to those derived numerically, as represented in Figure 6.4. The strongest factor determining the interepidemic interval here is again the force of infection, with calculated results using different waning rates exactly overlapping in Figure 6.5.

Another stabilizing influence in this system is the relatively long duration of infection. Analytic results were obtained for the interepidemic period, given a range of values of λ and υ for a fixed value of ω (arbitrarily fixed at 0.5).

Figure 6.6: Analytic solution for the interepidemic period, for different values of λ and υ



Here the interepidemic interval can be seen to vary in response to both the force and duration of infection. Again, the prevalence of infected and resistant individuals was calculated for the range of variables represented above, in order to determine which would produce results compatible with |UK population data.

Figure 6.7 Proportion of the population who are resistant

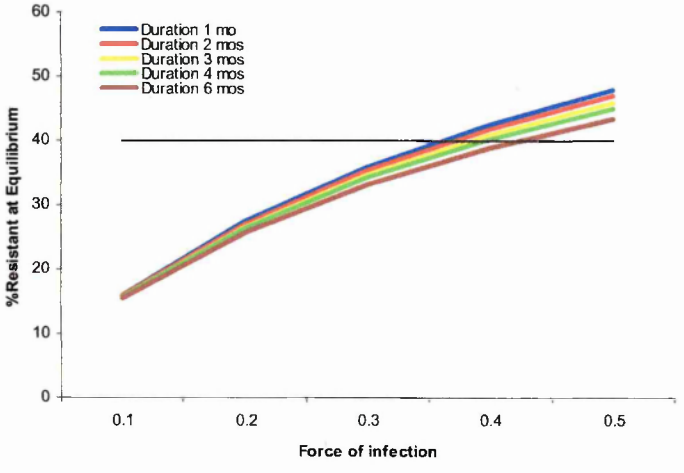
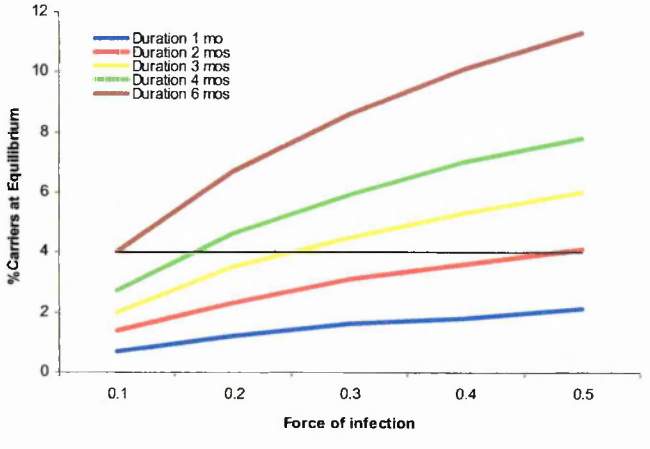


Figure 6.8 Proportion of the population who are infected



Here only the force of infection determines the proportion resistant, with an estimate of 0.4 resulting in 40% seroprevalence of protective antibody titres. The duration of carriage for this force of infection which would result in 4% prevalence of colonisation is between 2 and 3

months. Under these assumptions, the interepidemic interval is calculated to be between 5 ½ and 6 ½ years, which would have resulted in a peak rebound in all infections in 1998-1999. This result is not inconsistent with the model output shown in Figure 6.1 c) at the beginning of the chapter. As such, we believe that it is not unreasonable to deduce that the current rise in invasive Hib infections has resulted from a second wave of recurrence of circulation of the organism in the population, and that the first recurrence was 'hidden' due to the high antibody titres induced by the initial catch-up immunization campaign conducted in 1992/3.

6.3 Effect of changing mixing assumptions

All of the models in Sections 6.1 and 6.2 assumed uniformity of mixing and subsequent Hib transmission in the population. Based on what we know about the social context of children's daily lives, such a situation is unlikely to be true. The difficulty in parameterising contact patterns for the purposes of epidemiological models is the inability to quantify the true proportional significance of social interactions to infection risk and thus transmission coefficients between individuals of different ages, as discussed in Section 5.7. In consequence, it was necessary to explore the effect of imposing a range of contact matrices incorporating different assumptions about mixing between age groups in order to understand the sensitivity of model predictions to these differences.

Using the same baseline parameter set for the age structured model as in Section 6.1, a range of values for β were calculated, based on different contact mixing matrices. The consequences of vaccine introduction, with a catch-up campaign and use of poorly immunogenic vaccine, were then compared for these mixing assumptions to see which would result in levels of carriage and invasive disease consistent with the observed data. In Section 5.7, it was proposed that mixing would be strongly assortative, with most infections acquired from other

children in the same age group, or the age group just above. Model outputs for the following matrices were as shown:

a) Purely assortative mixing

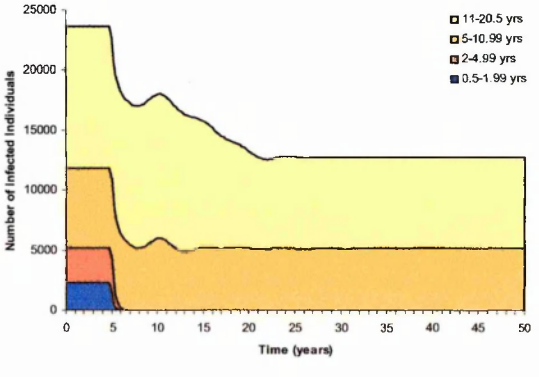
In this most extreme of mixing assumptions, children only acquired infections from others in the same age class.

$$\begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14} \\ C_{21} & C_{22} & C_{23} & C_{24} \\ C_{31} & C_{32} & C_{33} & C_{34} \\ C_{41} & C_{42} & C_{43} & C_{44} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

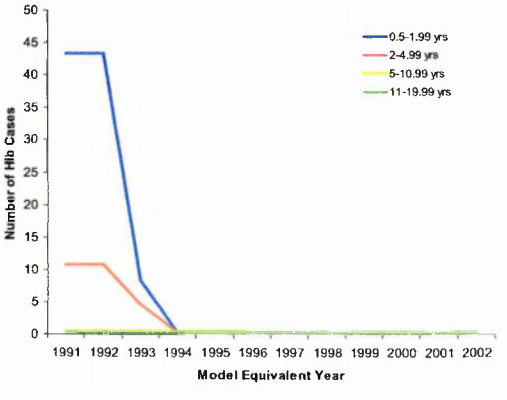
$$\beta_{11}=1.732 \times 10^{-4}, \beta_{22}=7.910 \times 10^{-5}, \beta_{33}=6.005 \times 10^{-5}, \beta_{44}=4.644 \times 10^{-5}$$

Figure 6.9: Number of infections, by age class and time, under a purely assortative mixing assumption

a) Carriage



b) Invasive Disease



Such a situation would lead to complete eradication of Hib from children under 5 years of age, which has not been the UK experience. This result highlights the important role of mixing with older age classes in driving ongoing infection in the most vulnerable age groups.

b) Social Mixing Assumption [1]

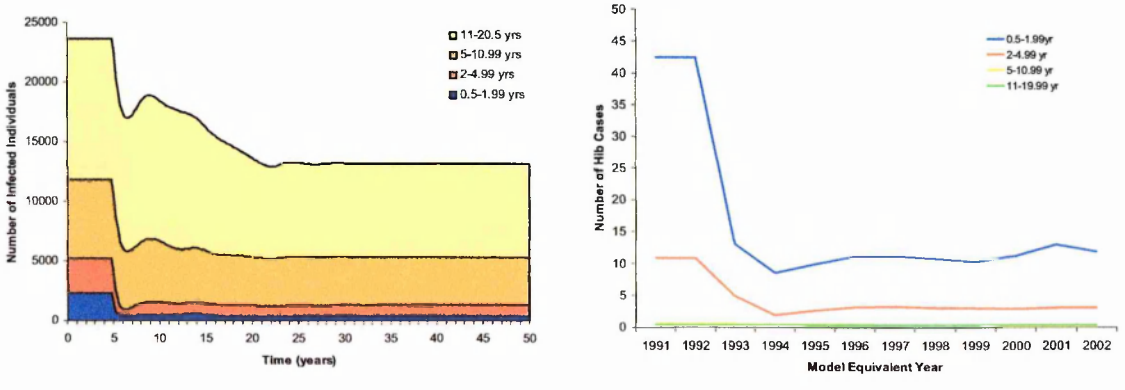
This matrix was strongly assortative, but assumed relatively large contributions of adjoining age classes to the number of infections experienced by children of a particular age group. As discussed in Section 5.7, mixing within the youngest age group of infants was assumed to be less than that observed within groups of older children, as many more would be being cared for in the home environment, and thus not in contact with large numbers of children in their own age class.

$$\begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14} \\ C_{21} & C_{22} & C_{23} & C_{24} \\ C_{31} & C_{32} & C_{33} & C_{34} \\ C_{41} & C_{42} & C_{43} & C_{44} \end{bmatrix} = \begin{bmatrix} 1 & 5 & 1 & 0 \\ 5 & 5 & 2 & 0 \\ 1 & 2 & 5 & 1 \\ 0 & 0 & 1 & 5 \end{bmatrix}$$

β_{11}	1.943E-05	β_{21}	2.735E-05	β_{31}	7.503E-06	β_{41}	0
β_{12}	9.713E-05	β_{22}	2.735E-05	β_{32}	1.501E-05	β_{42}	0
β_{13}	1.943E-05	β_{23}	1.094E-05	β_{33}	3.751E-05	β_{43}	8.348E-06
β_{14}	0	β_{24}	0	β_{34}	7.503E-06	β_{44}	4.174E-05

Figure 6.10: Number of infections, by age class and time, under mixing assumption [1]

a) Carriage **b) Invasive Disease**



This mixing matrix resulted in an insufficient reduction in the number of infections in the youngest individuals. In order to overcome this, a new assumption was made that mixing was more strongly assortative than previously supposed.

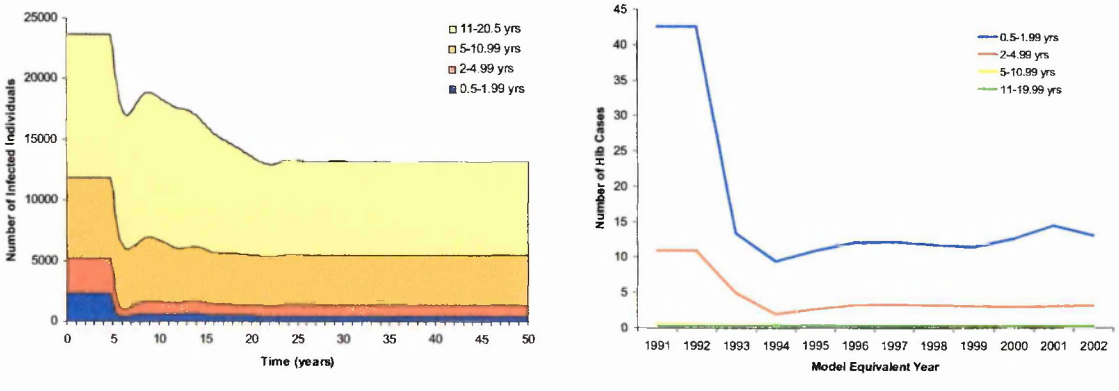
c) Social Mixing Assumption [2]

$$\begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14} \\ C_{21} & C_{22} & C_{23} & C_{24} \\ C_{31} & C_{32} & C_{33} & C_{34} \\ C_{41} & C_{42} & C_{43} & C_{44} \end{bmatrix} = \begin{bmatrix} 1 & 10 & 1 & 0.5 \\ 10 & 10 & 2.5 & 0.5 \\ 1 & 2.5 & 10 & 1 \\ 0.5 & 0.5 & 1 & 10 \end{bmatrix}$$

β_{11}	1.047E-05	β_{21}	2.881E-05	β_{31}	4.545E-06	β_{41}	2.152E-06
β_{12}	0.0001047	β_{22}	2.881E-05	β_{32}	1.136E-05	β_{42}	2.152E-06
β_{13}	1.047E-05	β_{23}	7.202E-06	β_{33}	4.545E-05	β_{43}	4.304E-06
β_{14}	5.233E-06	β_{24}	1.440E-06	β_{34}	4.545E-06	β_{44}	4.304E-05

Figure 6.11: Number of infections, by age class and time, under mixing assumption [2]

a) Carriage **b) Invasive Disease**



Both mixing assumptions [1] and [2] resulted in a less than observed reduction in the number of infections in the younger age classes, particularly noticeable in those less than 2 years of age. As a result, an even more strongly assortative mixing structure was imposed.

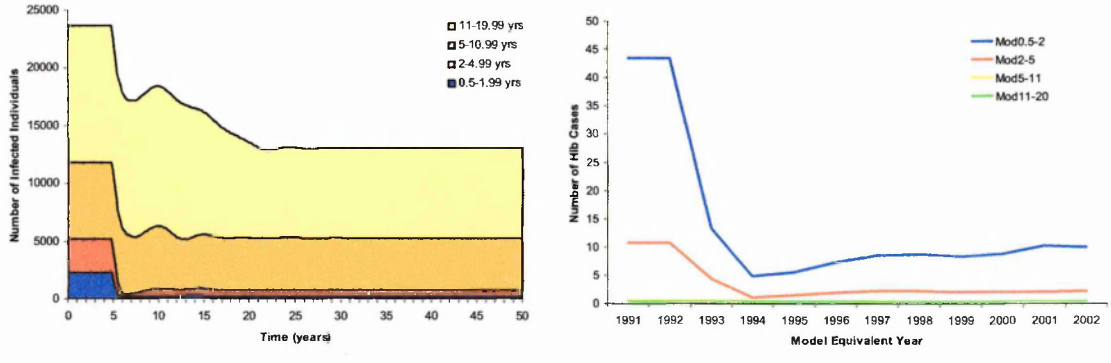
d) Social Mixing Assumption [3]

$$\begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14} \\ C_{21} & C_{22} & C_{23} & C_{24} \\ C_{31} & C_{32} & C_{33} & C_{34} \\ C_{41} & C_{42} & C_{43} & C_{44} \end{bmatrix} = \begin{bmatrix} 1 & 100 & 1 & 0.1 \\ 100 & 100 & 10 & 0.1 \\ 1 & 10 & 100 & 1 \\ 0.1 & 0.1 & 1 & 100 \end{bmatrix}$$

β_{11}	1.559E-06	β_{21}	3.632E-05	β_{31}	5.646E-07	β_{41}	4.616E-08
β_{12}	0.0001559	β_{22}	3.632E-05	β_{32}	5.646E-06	β_{42}	4.616E-08
β_{13}	1.559E-06	β_{23}	3.632E-06	β_{33}	5.646E-05	β_{43}	4.616E-07
β_{14}	1.559E-07	β_{24}	3.632E-08	β_{34}	5.646E-07	β_{44}	4.616E-05

Figure 6.12: Number of infections, by age class and time, under mixing assumption [3]

a) Carriage **b) Invasive Disease**



This model produced no late rebound in infections in 2-5 year olds arising from exposure to the age class above. On closer inspection, this was not surprising as the 2-5 year olds themselves exerted a relatively strong influence on acquisition of infections by the 5-11 year old group. Ecological studies of Hib infection implicated older school-aged siblings as an important risk factor for Hib disease in infants and toddlers, suggesting asymmetry of household transmission (Ounsted C 1950, 1951). In order to change the balance of power in the infectious relationship, the value of C_{32} was reduced from 10 to 1 in the following model. In addition, contact parameter values of 0.1, which were so negligible as to be insignificant in their effects, were changed to 0 for simplicity.

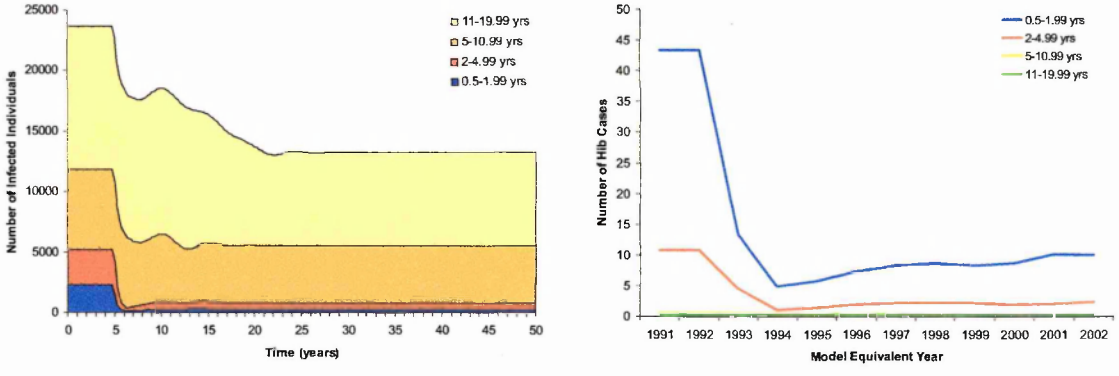
e) Social Mixing Assumption [4]

$$\begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14} \\ C_{21} & C_{22} & C_{23} & C_{24} \\ C_{31} & C_{32} & C_{33} & C_{34} \\ C_{41} & C_{42} & C_{43} & C_{44} \end{bmatrix} = \begin{bmatrix} 1 & 100 & 1 & 0 \\ 100 & 100 & 10 & 0 \\ 0 & 1 & 100 & 0 \\ 0 & 0 & 0 & 100 \end{bmatrix}$$

β_{11}	1.566E-06	β_{21}	3.639E-05	β_{31}	0	β_{41}	0
β_{12}	0.0001566	β_{22}	3.639E-05	β_{32}	5.980E-07	β_{42}	0
β_{13}	1.566E-06	β_{23}	3.639E-06	β_{33}	5.980E-05	β_{43}	0
β_{14}	0	β_{24}	0	β_{34}	0	β_{44}	4.644E-05

Figure 6.13: Number of infections, by age class and time, under mixing assumption [4]

a) Carriage **b) Invasive Disease**

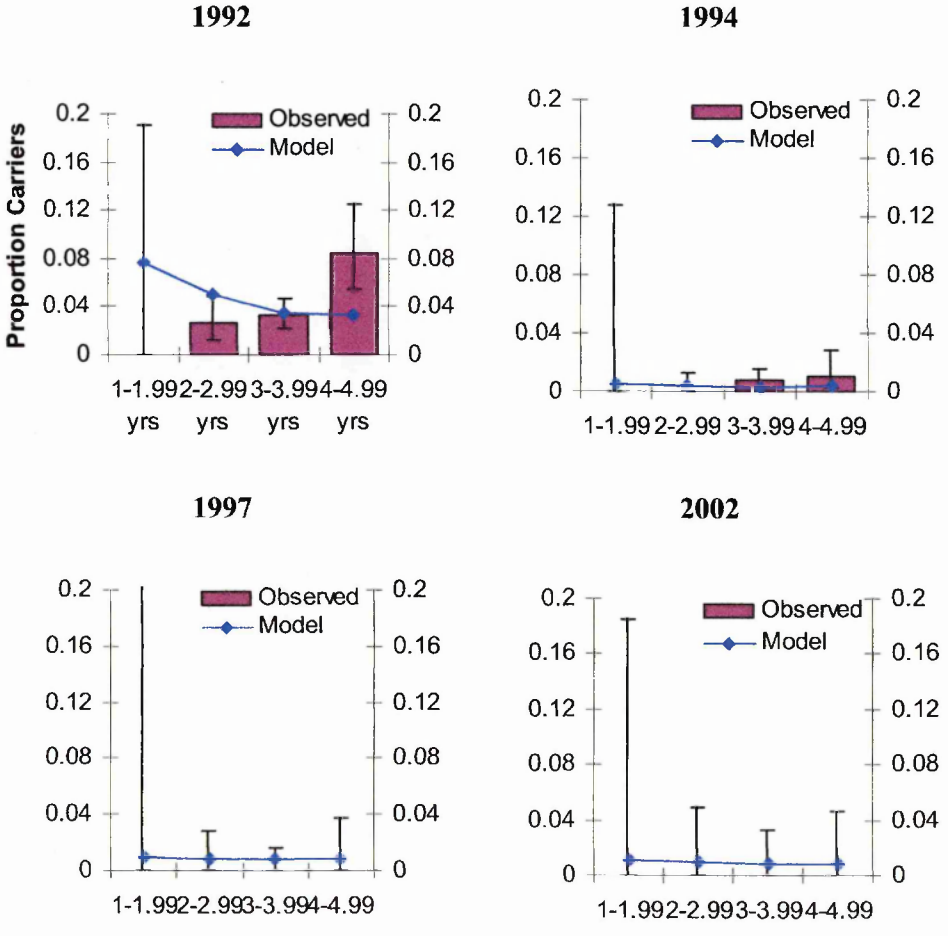


Social mixing assumptions [3] and [4] are associated with a more dramatic reduction in infections in the younger age classes after vaccine introduction, with a late rebound occurring from 1999 onwards. The latter model also results in an increase in invasive disease cases in 2-5 year olds from 2001. Under the baseline set of parameters, the number of cases which progress to invasive disease is, however, still too high.

The fit of this last model to the observed seroepidemiology and carriage data is shown in Figure 6.14. The carriage data is from the HPA nursery carriage study described in Section 2.5.1. The seroepidemiology data is from the same published study (Trotter CL et al 2003b) (Section 2.6.2) from which the pre-vaccination immunity data was derived.

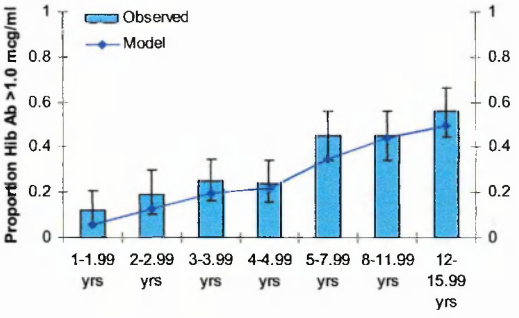
Figure 6.14: Social Mixing Assumption [4] – fit to observed data. In all of the figures below, columns represent observed population data, with error bars showing 95% confidence intervals of these estimates. Model outputs are represented by dots and lines.

a) English Nursery Carriage Dataset (1-4 year olds), by year and age

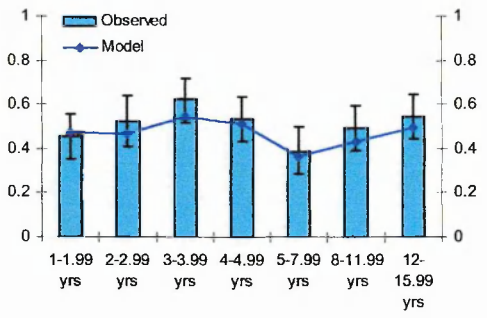


b) English Seroepidemiologic Data – High Level Immunity, by year and age

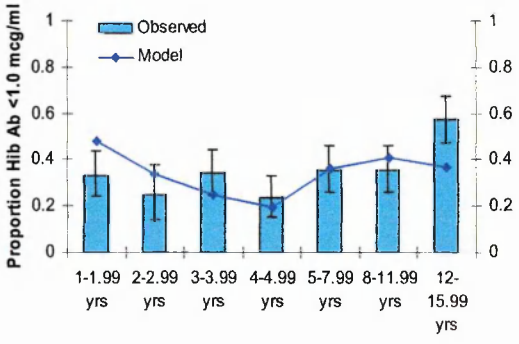
1990



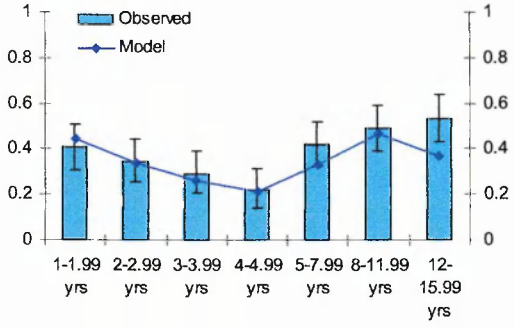
1994



1997

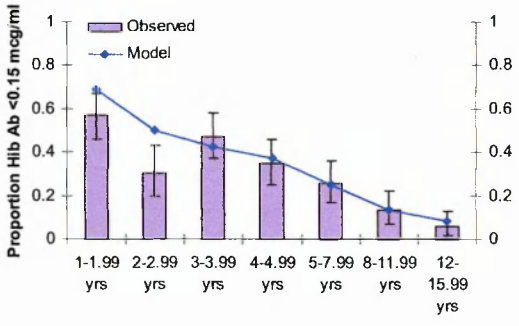


2000

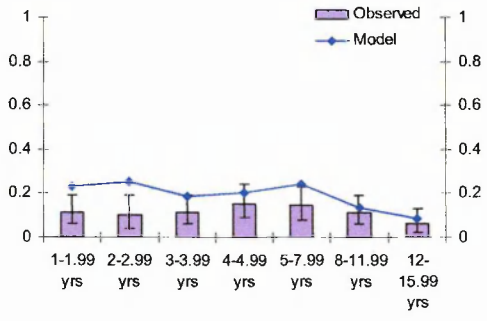


c) English Seroepidemiologic Data – No measurable immunity

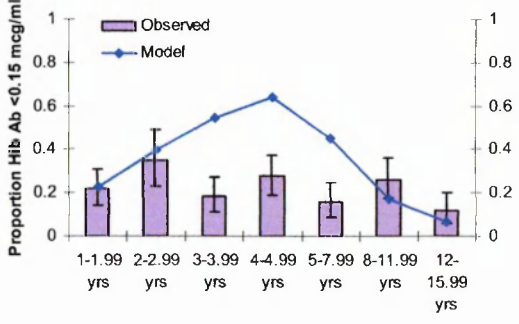
1990



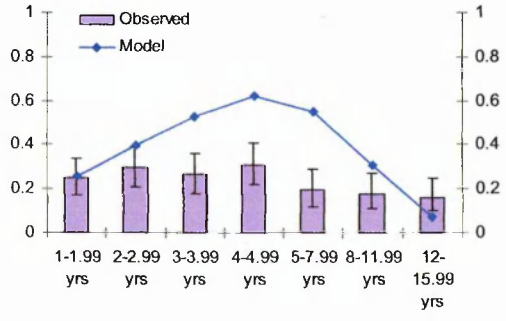
1994



1997



2000



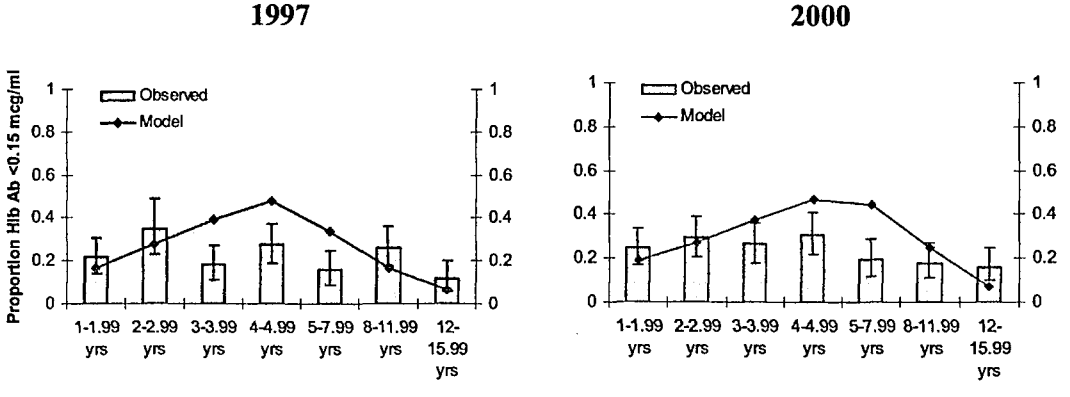
It can be seen that the model fits well to the observed carriage and high level immunity data. From 1997 onwards, however, the proportion estimated to have undetectable antibody levels is far in excess of that observed in the population. It was essential to correct this mismatch between model and data before moving on to test assumptions about vaccine protection against acquisition and progression to invasive disease from a state where individuals had been exposed, but immunity had waned. For this reason, the parameter ω_{LV}, determining the time period in which low measurable antibody levels were maintained, was subjected to sensitivity analysis.

6.4 Sensitivity analysis on duration of low immunity in the vaccinated proportion (ω_{LV})

When estimating pre immunisation parameters, it was noted that the duration of low level immunity following exposure to Hib was longer than would be predicted from the rate of waning of the acute phase antibody response, lasting 11 years (Section 5.3.2). The same would appear to be the case for vaccine induced immunity on the basis of Figure 6.15 c) above. This is likely to be due to ongoing antibody production by long lived plasma cells following waning of the primary immune response (Slifka MK et al 1998). A sensitivity analysis was conducted on the duration of low level antibody persistence.

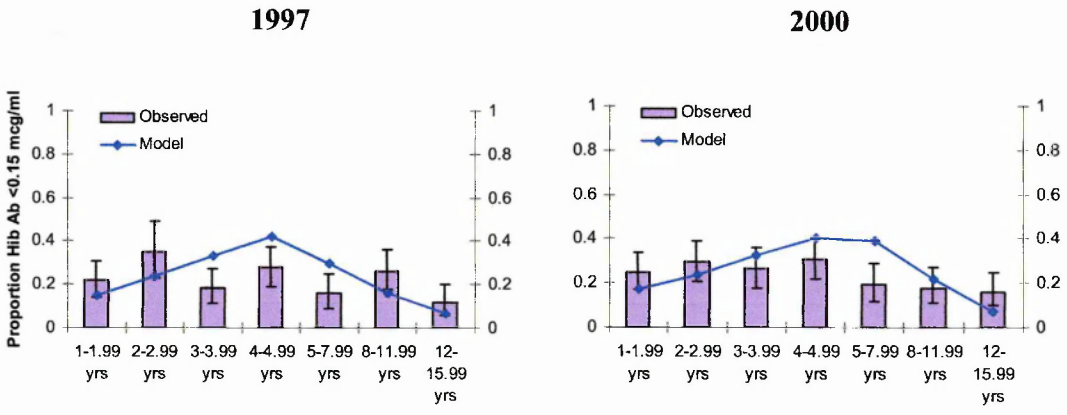
a) Low level immunity lasts for 3 years ($\omega_{LV} = 0.33$)

Figure 6.15: Undetectable immunity in England, 1997 & 2000, model fit if $\omega_{LV} = 0.33$



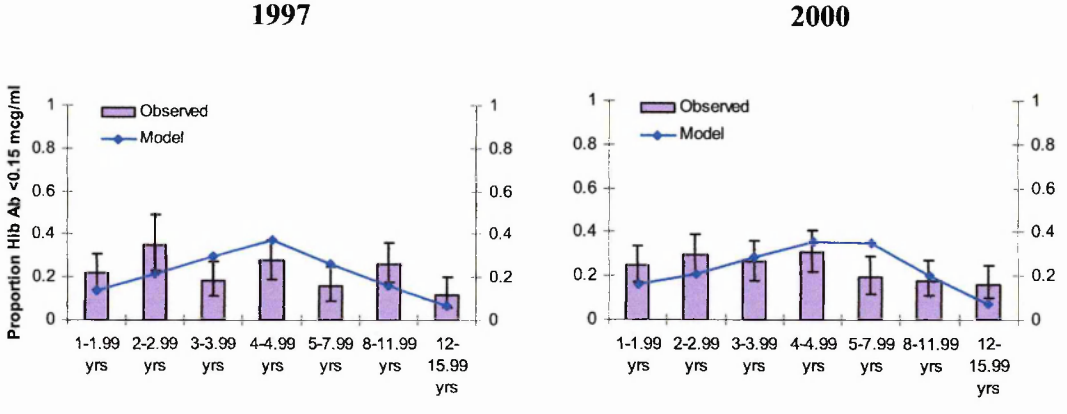
b) Low level immunity lasts for 4 years ($\omega_{LV} = 0.25$)

Figure 6.16: Undetectable immunity in England, 1997 & 2000, model fit if $\omega_{LV} = 0.25$



c) Low level immunity lasts for 5 years ($\omega_{LV} = 0.2$)

Figure 6.17: Undetectable immunity in England, 1997 & 2000, model fit if $\omega_{LV} = 0.2$



This improved fit to the population data on undetectable antibody titres did not compromise the model fit to any other observed data. On this basis, it was possible to go on to further explore assumptions regarding vaccine protection against acquisition of infection and progression of established infections to invasive disease. Subsequent models incorporated the new parameter value: $\omega_{LV} = 0.2$.

6.5 Sensitivity analysis on assumed protection against acquisition from the low immune state (ε)

The level of serum anticapsular antibody required to prevent Hib acquisition in vaccinated children has not been conclusively established (Section 1.15). Given this uncertainty, a sensitivity analysis was conducted in order to estimate the relative protection afforded by Hib titres between 0.15 and 1.0 g/ml over a range of values from 0 (no protection) to 1 (complete protection). Two consequences of increasing ε were:

(i) Increasing parameter values for the age dependent force of infection, λ.

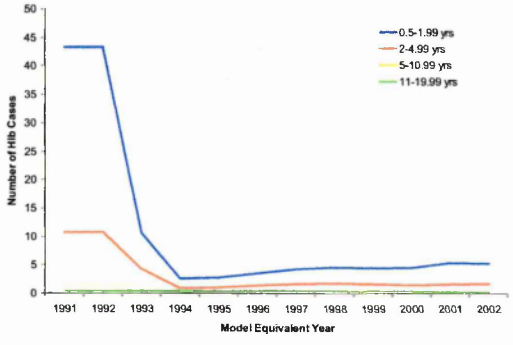
(ii) A relative increase in the proportion of all infections which were 'first infections'. As a result, in situations where ε was larger, log-likelihood estimation predicted improved protection against progression to invasive disease from the low immune and no measurable immunity (non-naïve) states, expressed in the parameters θ and μ respectively.

Model behaviour was explored over a range of these assumptions, to see which values would allow good initial control of infections, followed by the observed rebound in invasive disease incidence. As will be seen from Figures 6.18 to 6.20 below, increasing the value of ε, and subsequently of θ and μ, resulted in improved control of disease following vaccine introduction, but removed the phenomenon of the late increase in invasive disease observed from 1999 onwards. The only models which were able to reproduce this population behaviour were those which assumed very little protection against either acquisition or invasive disease from the low immune state. The direct protective efficacy of immunologic memory, represented by individuals in the S and S_V compartments, was thus estimated to be in the order of 35%. This figure is remarkably similar to the calculated direct protective efficacy of infant immunisation two years or more after vaccine administration in the UK, derived using the screening method (Ramsay ME et a; 2003).

a) Protection against acquisition from the low immune state, $\varepsilon = 0.25$, $\theta = 0.36$, $\mu = 0.36$

λ_1=0.47, λ_2=0.24, λ_3=0.47, λ_4=0.72

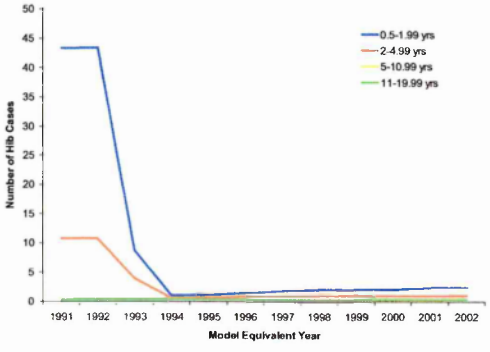
Figure 6.18: Invasive Disease Incidence Following Vaccine Introduction if $\varepsilon = 0.25$



b) Protection against acquisition from the low immune state, $\varepsilon = 0.5$, $\theta = 0.68$, $\mu = 0.68$

λ_1=0.48, λ_2=0.28, λ_3=0.57, λ_4=0.97

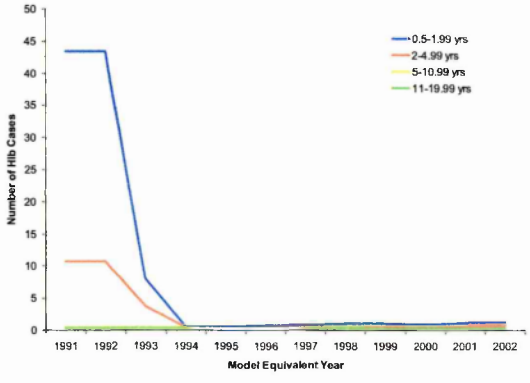
Figure 6.19: Invasive Disease Incidence Following Vaccine Introduction if $\varepsilon = 0.5$



c) Protection against acquisition from the low immune state, $\varepsilon = 0.75$, $\theta = 0.81$, $\mu = 0.81$

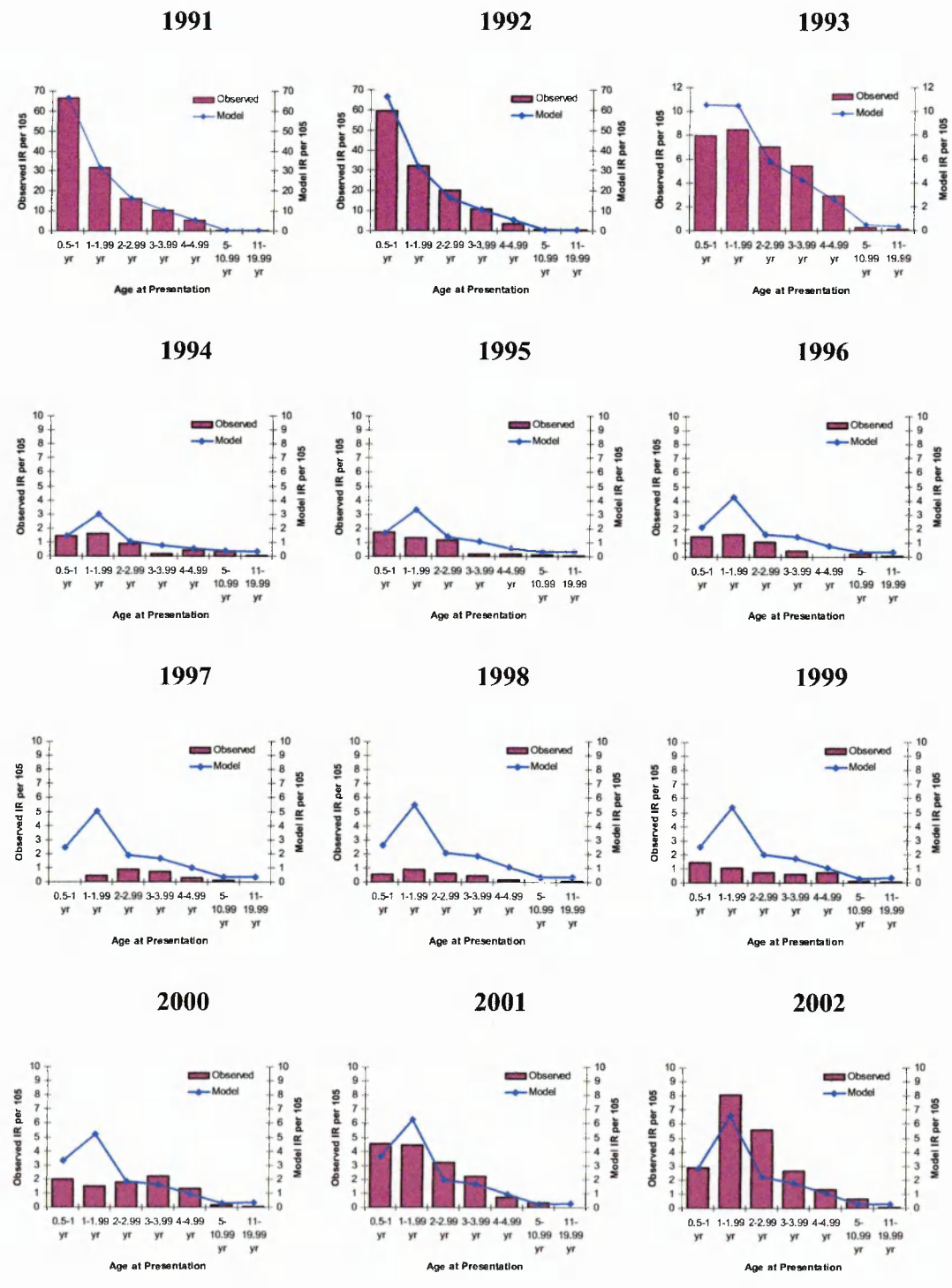
λ_1=0.50, λ_2=0.33, λ_3=0.71, λ_4=1.37

Figure 6.20: Invasive Disease Incidence Following Vaccine Introduction if $\varepsilon = 0.75$



Based on the results of the analysis illustrated in Figures 6.19-6.21, it was concluded that the protective efficacy of both low level antibody and the memory state were very low, as these were the only conditions under which a rebound of infections in the youngest age classes could occur. After exploring a range of potential values for ε in between those shown above, no better fit to the observed data was able to be achieved than was seen using the parameter value $\varepsilon = 0.25$. Figure 6.22 shows the year by year fit of the model to observed invasive disease incidence, by age at presentation, for this parameter value. Note the change in the scale of the y axis (Observed incidence rate per 10^5) between 1992 and 1993.

Figure 6.21: Observed and Model predicted Hib incidence by age and year, 1991-2002.



6.6 Discussion of discrepancies between observed data and model predictions

This model very accurately recaptures the immediate impact of vaccine introduction. Two major discrepancies between model and population behaviours remain, however.

6.6.1 Less effective control of disease by immunization in 1-2 year old children in the model between 1996 and 1999 than in the observed data

A number of possible reasons may contribute to this difference:

a) Homogeneous transmission of Hib is assumed between age classes deemed to mix with each other according to the contact matrix. Prior to vaccine introduction, carriage prevalence in the population was in the order of 4-5%. Even in this state, much higher pockets of transmission were noted, particularly in places such as nurseries, where many children mixed together. Following near eradication of carriage in the youngest age classes resulting from vaccine introduction, it may be erroneous to assume that reseeding of the organism within communities occurs in a uniform manner. Clustering of small groups of children in homes, playgroups and nurseries may have resulted in a slower increase in infections over time, as some groups would have the infection introduced, but not others. This effect would be anticipated to be less in schools, where larger numbers of children of many ages mix.

This hypothesis is supported by the findings of carriage studies conducted in nurseries over a ten year period (Section 2.5.1). A rapid reduction in Hib carriage prevalence was observed between 1992 and 1994, with no carriers detected among hundreds of children sampled in 1997 and 2000 (McVernon J et al 2003c). This did not mean that vaccinated children who had been exposed to Hib would not subsequently carry and transmit, however, as evidenced by an outbreak of invasive Hib infection among fully immunised children in a day care nursery in Northamptonshire (Section 2.5.2) (McVernon J et al 2003b). In this way, the inability of the

model to incorporate the phenomenon of non-uniform transmission across Britain may have led to an overestimate of carriage prevalence and hence invasive disease incidence in the years shortly after vaccine introduction.

A further example of such a phenomenon is given by the observed change in the epidemiology of measles which resulted from widespread vaccine introduction in England. Before immunisation was available, the timing of epidemics was strongly correlated between large cities. A reduction in the size of epidemics resulting from vaccination led to a breakdown of this previously observed relationship, with outbreaks occurring in geographically distinct locations at very different times (Bolker BM et al 1996).

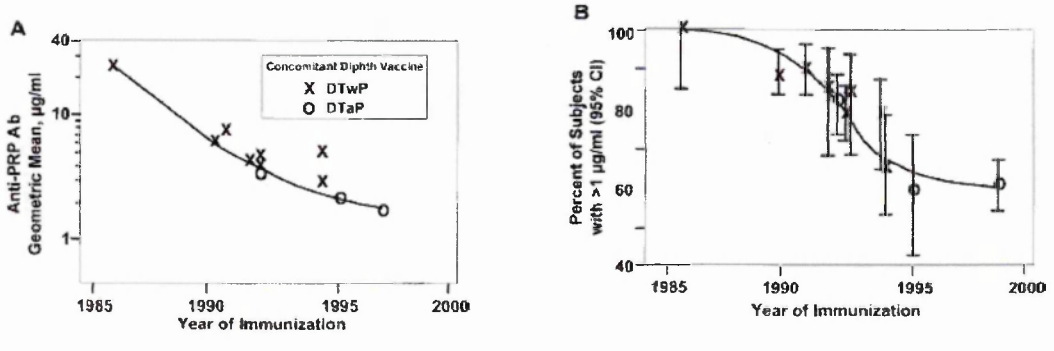
b) Prior to vaccine introduction, it is acknowledged that natural exposures resulting in the production of antibody with binding specificity to PRP may not all have been due to Hib, but also to cross-reactive organisms (Section 1.9). The pre-vaccination force of infection calculated for the youngest age group on the basis of observed immunity to Hib resulted in an estimated carriage prevalence of Hib rather higher than that observed in the population (Figure 5.4). It is likely that a proportion of these immunising contacts may have been due to other infections. As such, the calculated value for the transmission coefficient, β, in this age group may have been higher than warranted for Hib alone. As a result, the number of infections with Hib following vaccine introduction may have been selectively overestimated in this age category.

6.6.2 The recurrence of disease observed from 1999 onwards did not achieve the magnitude of the observed increase in England and Wales, although the distribution of ages at invasive disease was very similar.

The higher than predicted incidence of invasive disease in the UK from 2000 onwards may result from at least two further effects:

a) Vaccine efficacy may have truly diminished over time, resulting in more effective disease control in earlier years of the vaccination programme than that observed now. Evidence exists from the United States of declining immunogenicity of Hib conjugate vaccines over time. Dan Granoff describes a sequential reduction in post immunization antibody titres achieved following administration of the same single antigen Hib conjugate vaccine, measured using the same assay in aingle laboratory over a number of years, as shown in Figure 6.22 below (Granoff DM 2001). This reduction in immunogenicity remains unexplained on immunological grounds, but may represent a decrease in vaccine potency over time. No similar data is available from the UK.

Figure 6.22: Declining anti PRP antibody responses to *Haemophilus influenzae* type b oligosaccharides conjugated to CRM197 (HbOC) vaccine given as a separate injection to infants at 2, 4 and 6 months of age, concomitantly with DTaP or DTwP



Each point represents the value from a separate vaccine trial in which antibody concentrations obtained 1 month after the third dose were measured by a radioantigen-binding assay.

(A) Geometric mean antibody concentrations

(B) Percentage of infants (95% CI) with anti-PRP antibody concentrations >1.0 µg/ml.

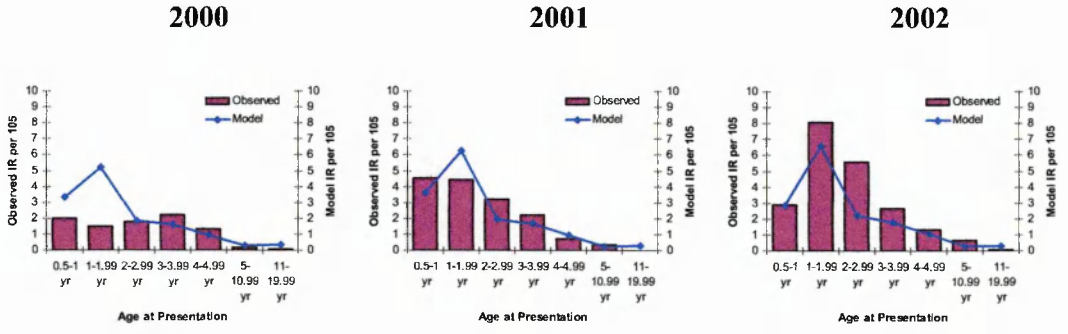
b) 2002 saw an increase in adult Hib disease cases to levels similar to or greater than those observed prior to vaccine introduction, as described in Section 2.4.3 (McVernon J et al 2004d). Transmission of Hib from adults to children is well described (Glode MP et al 1984). A lack of population based carriage and seroepidemiology data prevented inclusion of adults over 20 years of age in the model, but it appears likely that this may have resulted in omission of an important source pool of infections. Had adults been included in the model, it is likely that a greater increase in the force of infection would have been seen in the last few years than was noted in their absence.

In a similar scenario, in countries with established pertussis immunisation schedules, awareness of ongoing transmission of the disease among adolescents and adults raises question of the need for booster immunisation in these age groups (Fine PEM 1997). This issue has prompted study of a range of potential interventions to either boost immunity in the older cohort in order to provide direct protection for a large number, or to vaccinate parents as a 'cocoon' strategy aimed predominantly at indirect protection of infants (Van Rie A et al 2004).

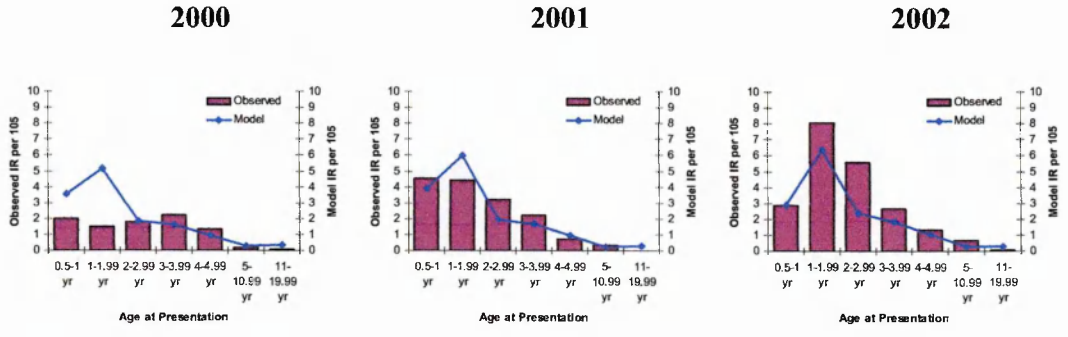
c) Approximately 20% of infants who received three doses of DTaP-Hib vaccine made no apparent antibody response by the end of the primary course. It has been previously assumed in the model that these children were all primed by vaccination, but it is possible that they may represent true primary vaccine failures. As such, this proportion of the 2000/2001 birth cohorts in the model may alternatively be considered to be fully naïve at entry (N), rather than contributing to the population of vaccinated modified susceptibles with no measurable antibody (S_V). The effect of changing this assumption is shown in the simulation below.

Figure 6.23: Effect of assuming that all vaccine 'non-responders' following three doses of DTaP-Hib are primary vaccine failures

a) Base case assumption



b) All non responders are primary vaccine failures



If all non-responders to primary immunisation are deemed to be primary vaccine failures, no significant increase in invasive disease incidence is observed in 2000-2002 compared with the base case (Figure 6.24). Given the low protective efficacy of prior exposure in the absence of measurable antibody in the model, this is perhaps not surprising. It was thus neither necessary nor feasible to invoke a high rate of primary vaccine failure to explain a late increase in invasive Hib infections in the UK.

6.7 Conclusions

The model as configured is highly parameterized, but has the benefit of being based on a large number of data points, using data of different types. Carriage, seroprevalence and invasive disease data have all been used to enable characterization of the relationships between Hib exposure, immunity and disease experience in the pre-vaccine condition. At baseline, 8 age specific estimates of carriage prevalence and 16 data points describing age specific immunity were used to define the age dependent forces of infection and waning rates. Age related progression to invasive disease was initially calculated against 7 age-specific measures of incidence.

Validation of model behaviour following introduction of immunization against ten years of observed population experience has allowed exploration of important theoretical assumptions regarding the mechanism and duration of vaccine protection. A further 16 serological data points were available for each of three years over this time (ie 48 measures of post-immunisation immunity). Carriage in children attending nursery in four age groups was available for comparison for four separate years (16 data points). Seventy measures of Hib disease incidence were available between 1993 and 2002 (7 age groups per year over 10 years), in order to assess the adequacy of parameter estimates of δ, ε, θ and μ.

Key theoretical conclusions of the model included a very low direct efficacy of immunologic memory following the primary course alone against disease, in keeping with estimates derived from the UK population using the screening method (Ramsay ME et al 2003). Minimal protection against acquisition of Hib was also observed in individuals with low-level measurable immunity. The age dependence of progression to invasive disease was in excess of the risk conferred by the observed absence of anti-polysaccharide antibodies, and could not

be solely explained by an increased risk of bacterial invasion associated with the first ever exposure.

Given these findings, the use of poorly immunogenic vaccines in 2000/2001 placed infants and young children at substantial risk of invasive Hib infections. According to the time scale predicted by the model, the increase in the size of the susceptible pool at this time was superimposed upon the second phase of a rebound in infections in older children associated with transients induced by catch-up immunisation in 1992/1993. While enormously successful at producing rapid disease control, catch-up campaigns such as this can be anticipated to induce marked fluctuations in transmission in the longer term. It was reassuring to note that the intrinsic properties of the system were likely to result in strong damping of these fluctuations, producing a new stable equilibrium within a relatively short time.

The model highlights the need for a greater understanding of the impact of immunisation on circulation of organisms in the adult population. Adults were not included in the model, due to an absence of baseline data upon which to parameterise the force of infection. A return of Hib disease incidence in this age group to pre-vaccine levels in recent years (Section 2.4.3) has potentially provided another source population likely to transmit to infants, further fuelling the rise in observed cases.

On the basis of the above analyses, the following final parameter values were used for the assessment of possible public health interventions on the ongoing epidemiology of Hib infections in Chapter 7.

Table 6.1: Final parameter set for use in the public health model

Model Parameter	Mean Value	Mean duration in class
Demographic parameters		
Births per quarter, B	6 250	
Population size, N	500 000	
Proportions vaccinated, p, q, z	0-1 where $p+q+z \leq 1$	
Routine infant immunisation	p=0.83, q=0.07, z=0.02	
Poorly immunogenic vaccine	p=0.63, q=0.19, z=0.10	
Biological parameters		
Duration of carriage for first infection		
υ'	5	2.4 months
Duration of carriage for subsequent infections		
υ (unvaccinated)	5	2.4 months
υ_V (vaccinated)	5	2.4 months
Duration of immunity		
Unvaccinated		
ω_{H1} (0.5-1.99 years)	6.36	0.16 years
ω_{H2} (2-4.99 years)	0.67	1.50 years
ω_{H3} (5-10.99 years)	0.46	2.18 years
ω_{H4} (11-20.5 years)	0.51	1.96 years
ω_L (all ages)	0.12	8.06 years
Vaccinated		
ω_{HV1} (0.5-0.99 years)	0.80	1.25 years
ω_{HV2} (1-1.49 years)	0.56	1.79 years
ω_{HV3} (1.5-1.99 years)	0.48	2.08 years
ω_{HV4} (2-20.5 years)	0.43	2.33 years
ω_{LV} (all ages)	0.20	5.0 years
Duration of immunity following re-exposure to Hib in vaccinated individuals		
ω_{B1} (0.5-0.99 years)	0.43	2.33 years
ω_{B2} (1-1.49 years)	0.57	1.75 years
ω_{B3} (1.5-1.99 years)	0.69	1.45 years
ω_{B4} (2-20.5 years)	0.95	1.05 years
Relative protection against acquisition from low antibody state		
ε	0.25	
Force of infection, λ		
λ_1 (0.5-1.99 years)	0.47	
λ_2 (2-4.99 years)	0.24	
λ_3 (5-10.99 years)	0.47	
λ_4 (11-20.5 years)	0.72	

Model Parameter	Mean Value	Mean duration in class
Rate of progression to invasive disease, δ		
δ_1 (0.5-0.99 years)	0.0118	
δ_2 (1.0-1.99 years)	0.0044	
δ_3 (2.0-2.99 years)	0.0044	
δ_4 (3.0-3.99 years)	0.0039	
δ_5 (4.0-4.99 years)	0.0021	
δ_6 (5.0-20.5 years)	0.00009	
Protection against progression against invasive disease afforded by low levels of measurable antibody, θ		
θ	0.36	
Protection against progression against invasive disease afforded by previous natural or vaccine exposure to Hib in the absence of measurable antibody, μ		
μ	0.36	

Chapter 7: Public Health Implications of the Model

7.0 Introduction

The implications of a range of possible changes to the UK vaccine schedule were explored using the model developed in Chapter 5. In attempting to simulate the effects of these changes, the considerable variation in immunogenicity observed between vaccine trials made it difficult to fix upon definitive values for the parameters describing the duration of protection following boosting ($\omega_{B1\text{-}4}$).

Results of immunisation trials using accelerated (2, 3, 4 months) and standard (2, 4, 6 months) schedules combining PRP-T with either DTwP or DTaP were compared. Only those incorporating a booster phase were included. Post primary and post boost (with conjugate or PRP) titres from a range of studies are shown in Figure 7.0 and Table 7.0.

Figure 7.0: Prime/boost titres achieved in Phase II trials using different vaccines and schedules (country of study shown). PRP denotes polysaccharide booster.

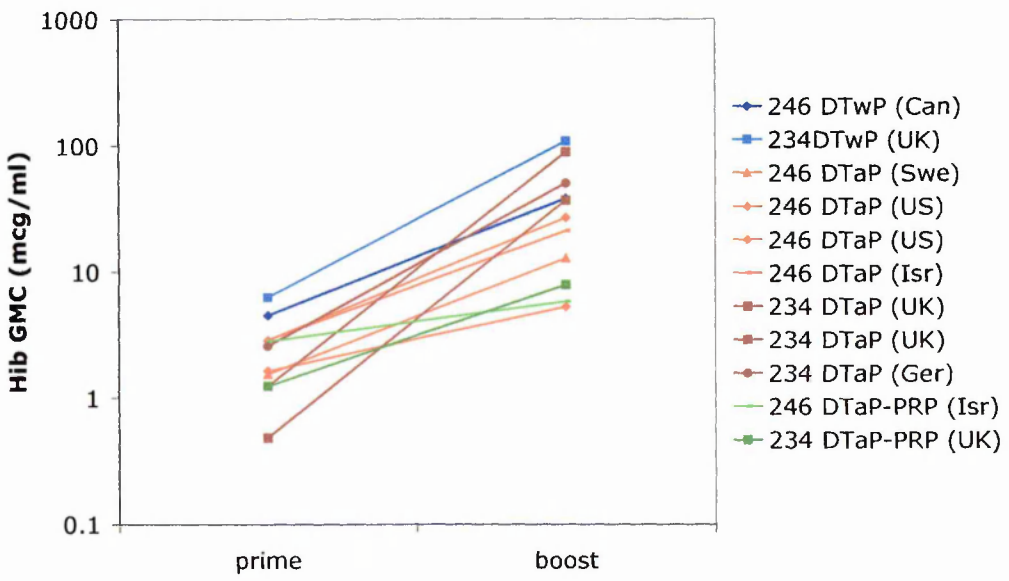


Table 7.0: Summary of immunogenicity trials combining prime/boost phase by schedule and vaccine

Study	Schedule	Post Primary Hib GMC µg/ml (95% CI)	% >1.0 µg/ml	% <0.15 µg/ml	Post Booster Hib GMC µg/ml (95% CI)	% >1.0 µg/ml	% >0.15 µg/ml
DTwP-Hib Combinations							
Scheifele D et al 1993/5 (Canada)	2, 4, 6 mo Conjugate boost 18-19 mo	4.50 (3.46, 5.86)	87%	3%	38.1 (32,3, 44.9)	100%	0%
Goldblatt D et al 1998 (UK)	2, 3, 4 mo Conjugate boost 1 year	6.23 (5.53, 7.01)	93%	2%	108.39 (91.62, 128.23)	98%	2%
DTaP-Hib Combinations							
Carlsson R-M et al 1998 (Sweden)	2, 4, 6 mo Conjugate boost 13 mos	1.53	67%	8%	12.75	99%	0%
Greenberg DP et al 2000 (US)	2, 4, 6 mo Conjugate boost 11-15 mo	1.63	71%	12%	5.26	100%	0%
Poolman J et al 2001 (US)	2, 4, 6 mo Conjugate boost 15-18 mo	2.84	86%	0%	26.8	100%	0%
Dagan R et al 2001 (Israel)	2, 4, 6 mo Conjugate boost 12 mo	2.86 (1.77, 4.63)	N/A	3%	21.09 (13.5, 32.94)	N/A	0%
Bell F et al 1998 (UK)	2, 3, 4 mo Conjugate boost 13 mos	0.48 (0.41, 0.57)	27%	18%	36.8 (21.0, 64.4)	97%	3%
Goldblatt D et al 1999 (UK)	2, 3, 4 mo Conjugate boost 12-15 mo	1.23 (0.98, 1.58)	82%	7%	88.5 (64.4, 121.5)	100%	0%
Poolman J et al 2001 (Ger)	2, 3, 4 mo Conjugate boost 12-18 mo	2.56	75%	1%	50.2	98%	0%
Dagan R et al 2001 (Israel)	2, 4, 6 mo Plain PRP boost 12 mo	2.81 (1.66, 4.84)	N/A	5%	5.81 (3.25, 10.39)	N/A	0%
Goldblatt D et al 1999 (UK)	2, 3, 4 mo Plain PRP boost 12-15 mo	1.23 (0.98, 1.58)	82%	7%	7.86 (5.30, 11.70)	85%	0%

In examining these data, several consistent findings may be observed in spite of considerable inter-study variation:

(i) Acceleration of the schedule does not have an adverse effect on either post primary or post-boost immunogenicity when DTwP is combined with PRP-T.

(ii) While accelerated schedules using DTaP have been associated with lower post-primary immunisation titres than standard courses in one vaccine manufacturer's summary (Vidor E et al 2001), similar ranges of immunogenicity results are seen when multiple trials are compared (Table 7.0). Interestingly, higher post boost titres are consistently observed following priming with the 2, 3, 4 month infant regimen (Figure 7.0).

(iii) Boosting with conjugate vaccine usually results in antibody concentrations 5-10 fold higher than are observed following polysaccharide boosting. Again, however, results vary widely following the fourth dose of conjugate. A log difference may be observed between the highest and lowest titres in the studies above, ranging from 12.75 μg/ml (Carlsson RM et al 1998) to 108.39 μg/ml (Goldblatt D et al 1998). The lowest values observed are in the order expected of those seen with primary immunisation in the second year of life, hardly corresponding to a 'boost' response at all.

7.1 Approach to examination of public health issues

On the basis of the data reviewed in section 7.0, the following approach was taken to the exploration of public health implications of the model. All simulations incorporated the catch-up immunisation campaign employed in the UK in 1992/1993 and the use of less immunogenic vaccines in 2000/2001, as described in Section 6.1.

(i) No justification could be seen for comparing a 2, 4, 6 month priming schedule with the currently used 2, 3, 4 month regimen.

(ii) The model in Chapter 5 assumed a post-boost titre for all vaccine recipients of 100 μg/ml, based on the response to carriage observed in three pre-school aged children in a single published study (Barbour ML et al 1993). It was thus tacitly assumed that exposure to an intact organism was more immunogenic than plain polysaccharide. This assumption may have been an overestimate, even when considering antibody titres achieved following boosting with conjugate vaccines. For this reason, a series of models were run and the resulting effects on disease incidence over time compared if the peak post boost response was 15, 50 or 100 μg/ml. Parameter values for ω_B by age group were calculated using the same methods shown in Section 5.3.4. Results are shown in Table 7.1.

(iii) The anticipated effect of a routine booster with 80% uptake in the previously immunised population, used from the time of implementation in 1992, was assessed for each of the post boost assumptions. The booster was scheduled at age 12 months, 15 months, 18 months or 4 years.

(iv) The effect of a one-off catch-up immunisation campaign, as employed in the UK in 2003, was explored given each of the three post boost assumptions, for coverage estimates of 20, 40, 60 and 80%.

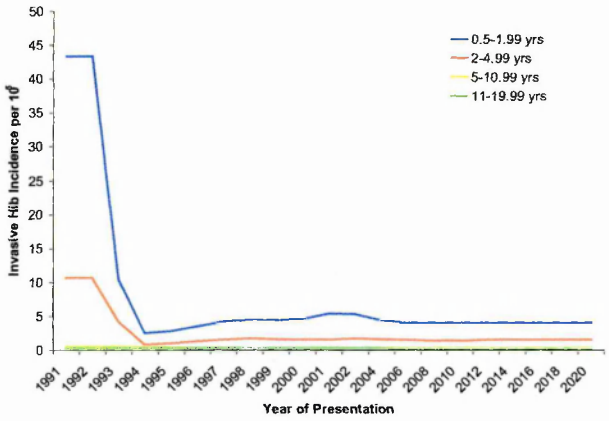
(v) The effect of a one-off catch-up immunisation campaign as employed in the UK in 2003, accompanied by introduction of a routine booster with 80% uptake in the previously immunised population, was explored given each of the three post boost assumptions, for catch-up coverage estimates of 20% and 60%. The booster was scheduled at either age 12 or 18 months, based on the timings found to be optimal in part (iii).

Table 7.1: Calculation of ω_B by age group, given different post boost titres achieved

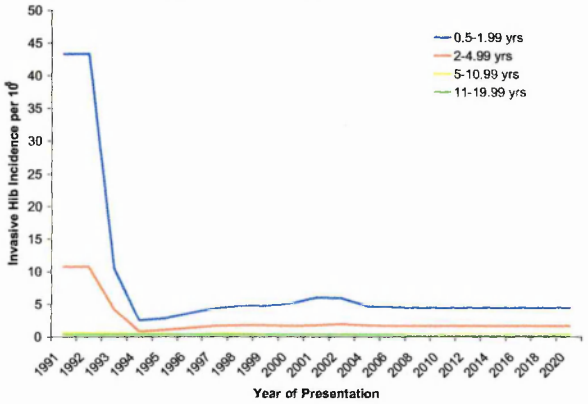
Mean post boost titre (μg/ml)			100		50		15	
Age group	**Parameter estimated**	**Post prime Ab response**	**Time to wane**	**Parameter value**	**Time to wane**	**Parameter value**	**Time to wane**	**Parameter value**
0.5-1.99 years	ω_{B1}	5.0	2.3 years	0.43	1.77 years	0.56	0.85 years	1.18
1-1.49 years	ω_{B2}	10.0	1.77 years	0.56	1.24 years	0.81	0.31 years	3.21
1.5-1.99 years	ω_{B3}	15.0	1.46 years	0.69	0.93 years	1.08	0 years	365
≥2 years	ω_{B4}	25.0	1.07 years	0.94	0.53 years	1.88	0 years	365

7.2 Model outputs assuming different magnitudes of boosting following natural exposure in immunised children

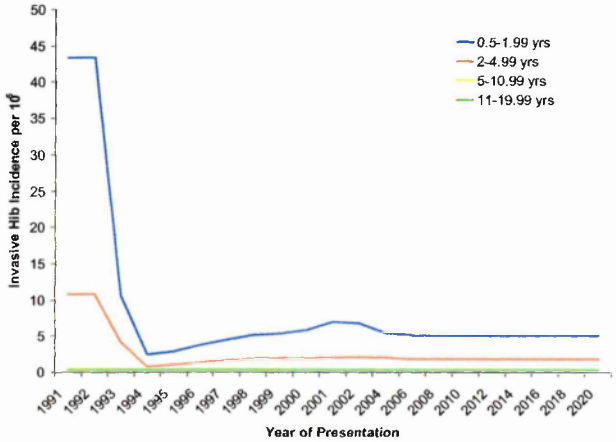
7.2.1 Boost response to natural exposure = 100 µg/ml



7.2.2 Boost response to natural exposure = 50 µg/ml



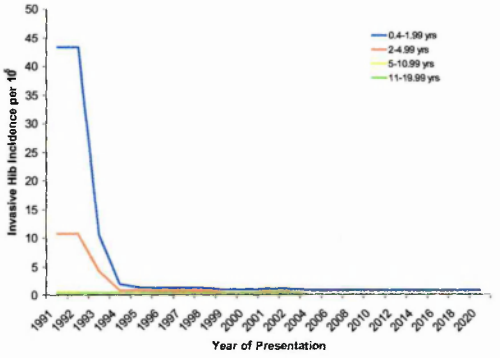
7.2.3 Boost response to natural exposure = 15 µg/ml



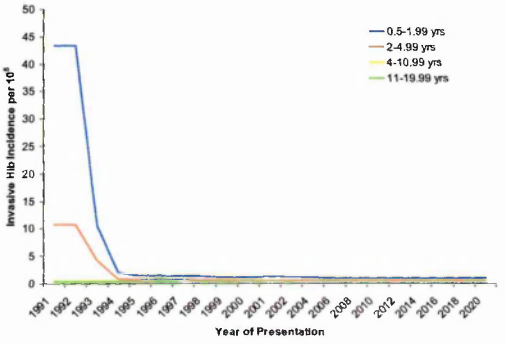
7.3 Model outputs assuming 80% uptake of a routine booster dose from 1993 onwards, at various ages and for different magnitudes of boost response

7.3.1 Boost response to vaccine or natural exposure = 100 µg/ml

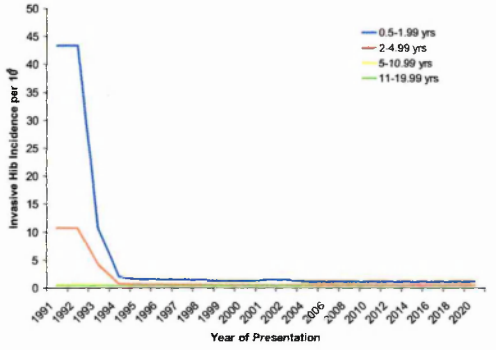
(i) Boost at 12 months



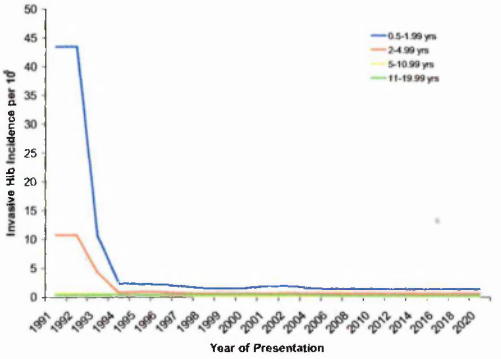
(ii) Boost at 15 months



(iii) Boost at 18 months

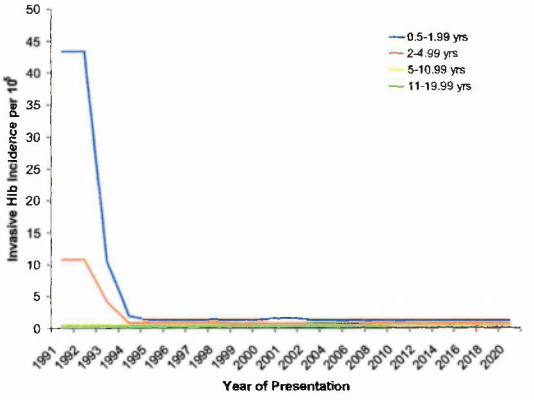


(iv) Boost at 4 years

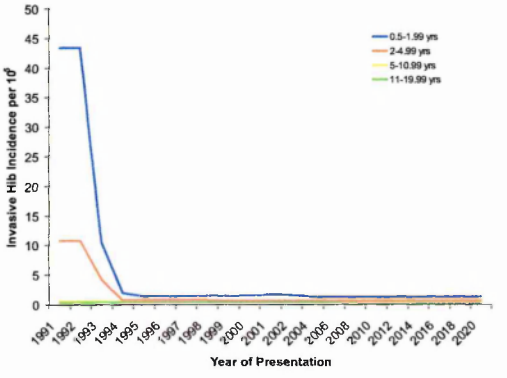


7.3.2 Boost response to vaccine or natural exposure = 50 μg/ml

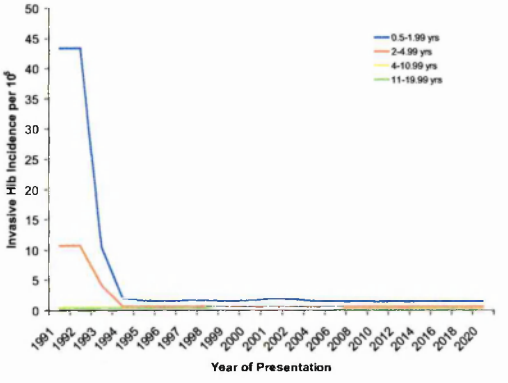
(i) Boost at 12 months



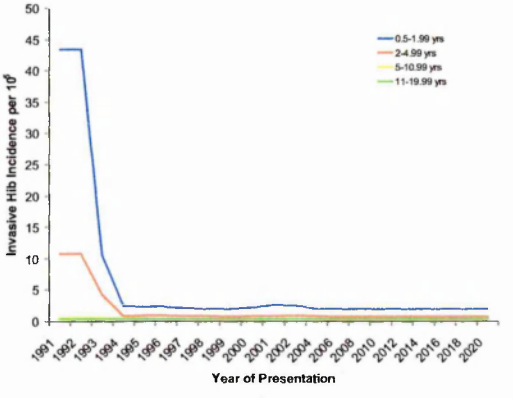
(ii) Boost at 15 months



(iii) Boost at 18 months

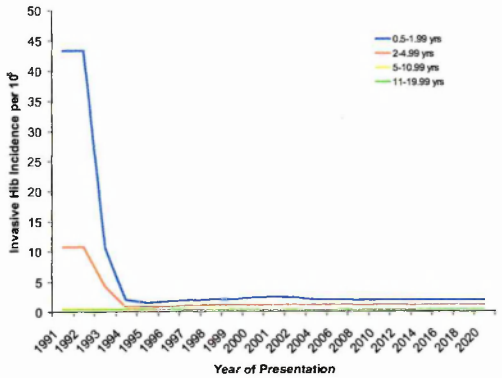


(iv) Boost at 4 years

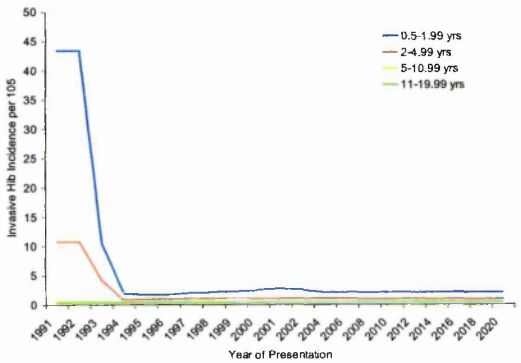


7.3.3 Boost response to vaccine or natural exposure = 15 µg/ml

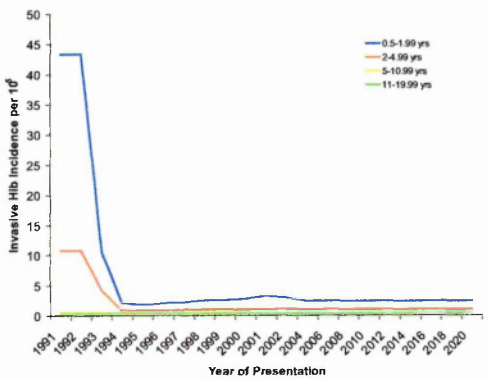
(i) Boost at 12 months



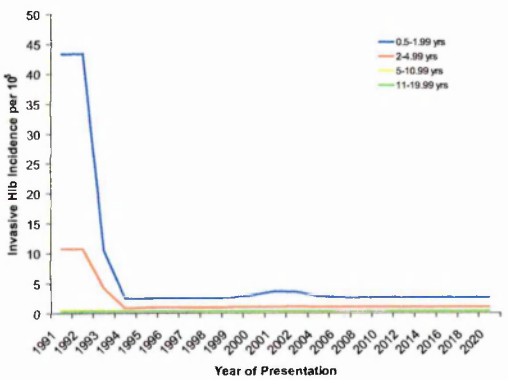
(ii) Boost at 15 months



(iii) Boost at 18 months



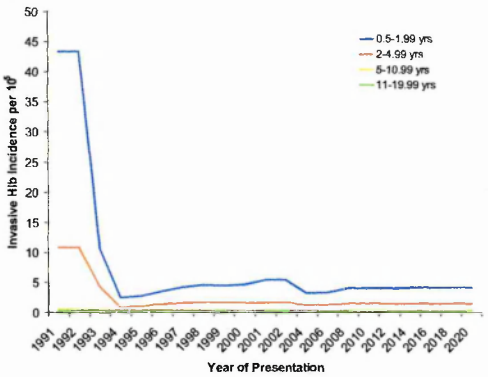
(iv) Boost at 4 years



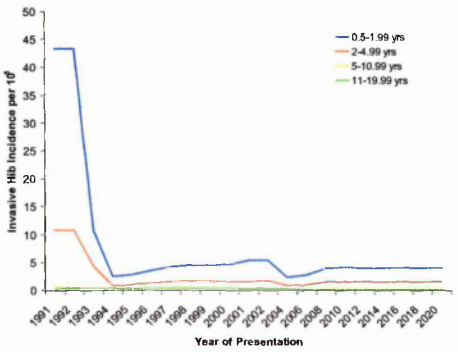
7.4 Model outputs assuming various levels of uptake of the 2003 catch-up immunisation campaign, for different magnitudes of boost response

7.4.1 Boost response to vaccine or natural exposure = 100 μg/ml

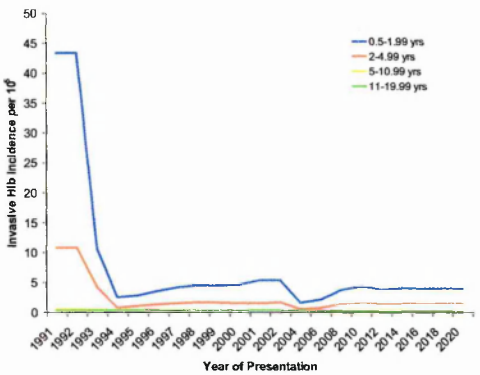
(i) 20% uptake of catch-up dose



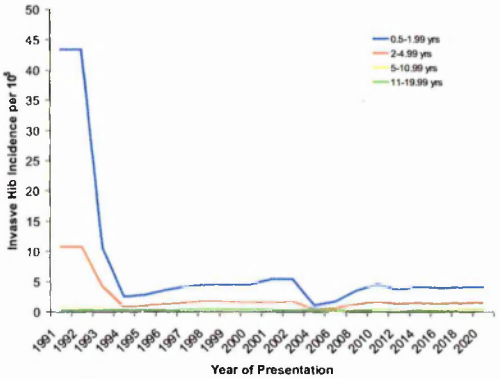
(ii) 40% uptake of catch-up dose



(iii) 60% uptake of catch-up dose

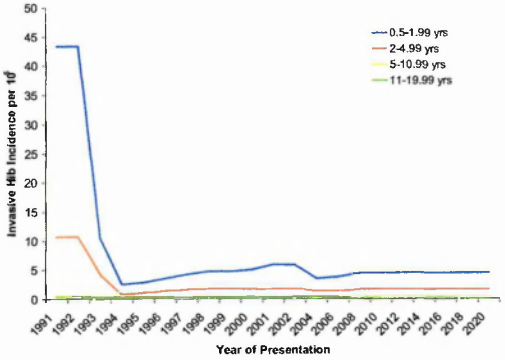


(iv) 80% uptake of catch-up dose

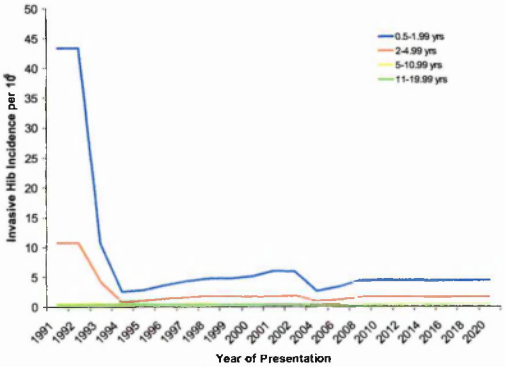


7.4.2 Boost response to vaccine or natural exposure = 50 μg/ml

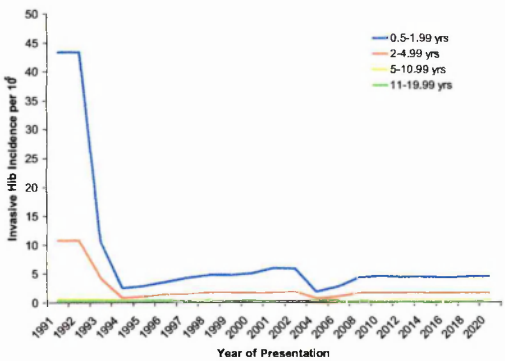
(i) 20% uptake of catch-up dose



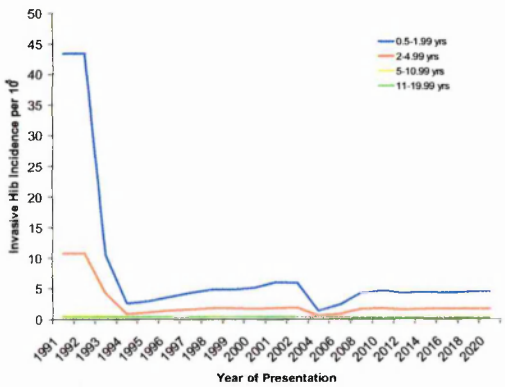
(ii) 40% uptake of catch-up dose



(iii) 60% uptake of catch-up dose

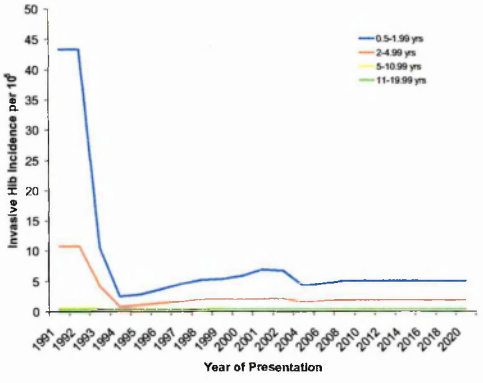


(iv) 80% uptake of catch-up dose

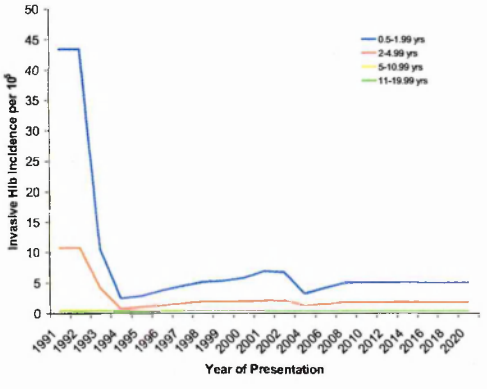


7.4.3 Boost response to vaccine or natural exposure = 15 µg/ml

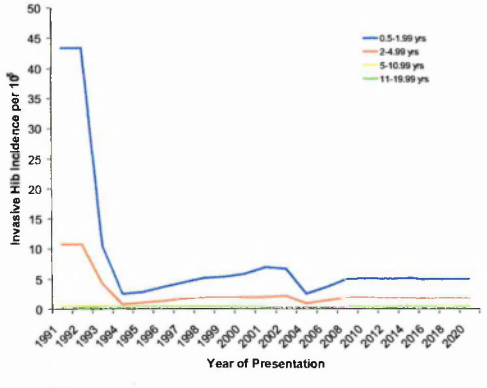
(i) 20% uptake of catch-up dose



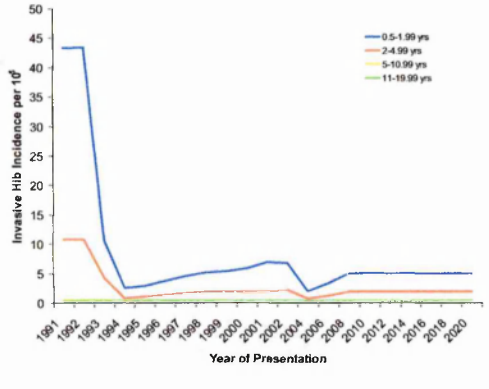
(ii) 40% uptake of catch-up dose



(iii) 60% uptake of catch-up dose



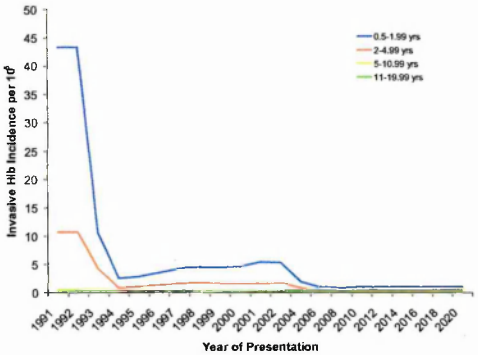
(iv) 80% uptake of catch-up dose



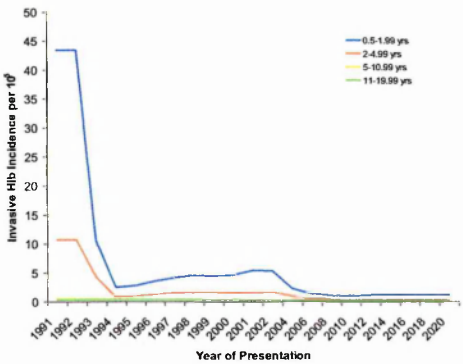
7.5 Model outputs assuming various levels of uptake of the 2003 catch-up immunisation campaign, accompanied by introduction of a routine fourth dose of vaccine

7.5.1 Boost response to vaccine or natural exposure = 100 µg/ml

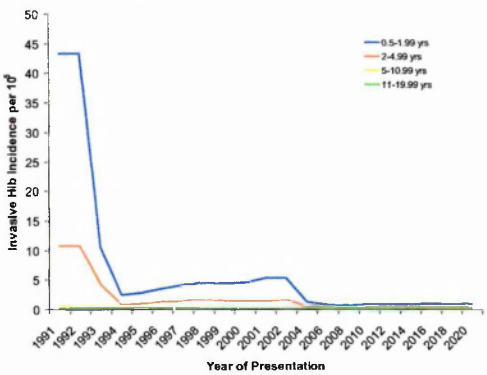
(i) 20% uptake of catch-up dose, with introduction of routine 12 month boost



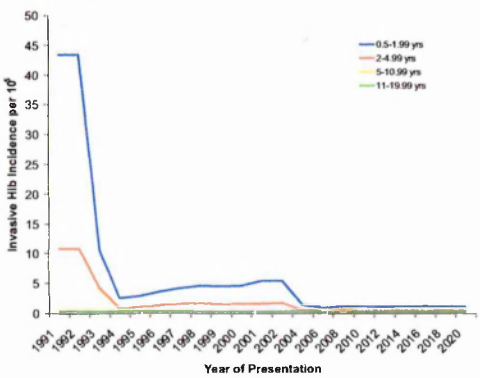
(ii) 20% uptake of catch-up dose, with introduction of routine 18 month boost



(iii) 60% uptake of catch-up dose, with introduction of routine 12 month boost

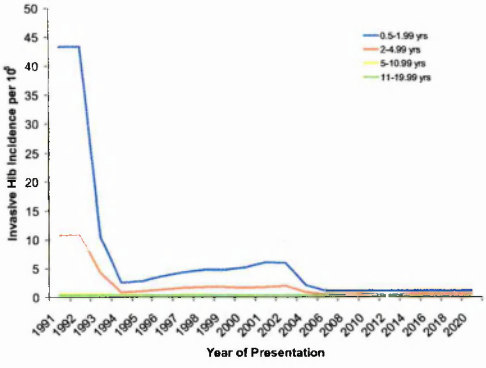


(iv) 60% uptake of catch-up dose, with introduction of routine 18 month boost

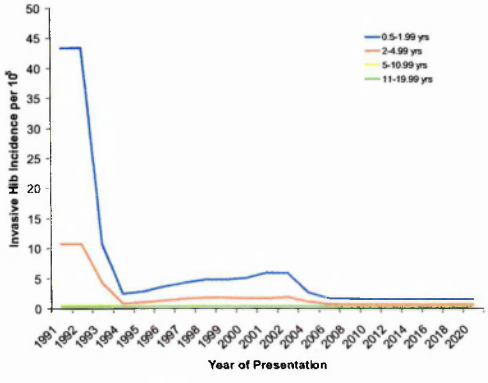


7.5.2 Boost response to vaccine or natural exposure = 50 µg/ml

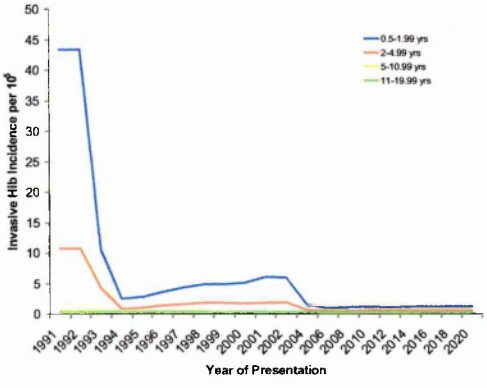
(i) 20% uptake of catch-up dose, with introduction of routine 12 month boost



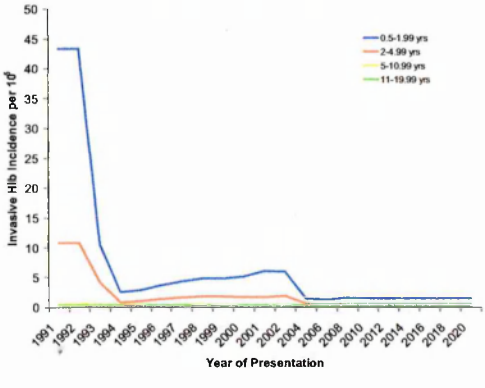
(ii) 20% uptake of catch-up dose, with introduction of routine 18 month boost



(iii) 60% uptake of catch-up dose, with introduction of routine 12 month boost

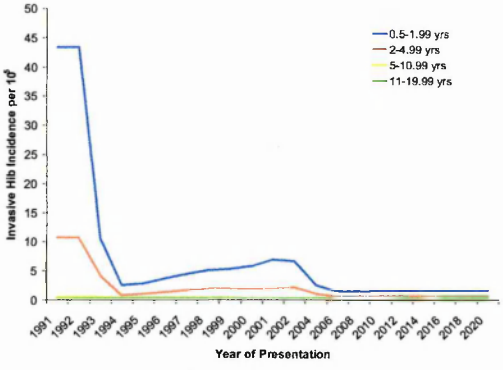


(iv) 60% uptake of catch-up dose, with introduction of routine 18 month boost

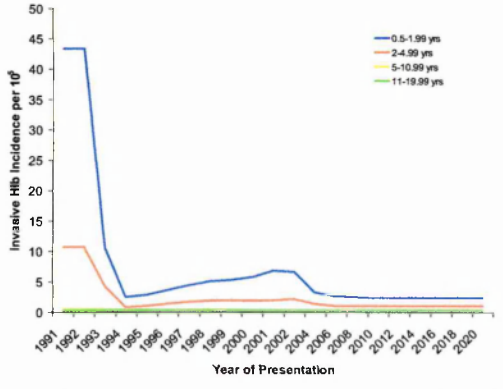


7.5.3 Boost response to vaccine or natural exposure = 15 μg/ml

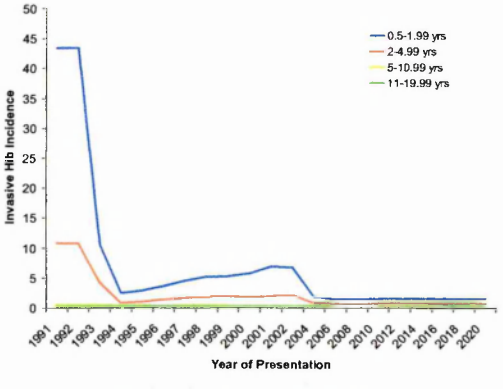
(i) 20% uptake of catch-up dose, with introduction of routine 12 month boost



(ii) 20% uptake of catch-up dose, with introduction of routine 18 month boost



(iii) 60% uptake of catch-up dose, with introduction of routine 12 month boost



(iv) 60% uptake of catch-up dose, with introduction of routine 18 month boost

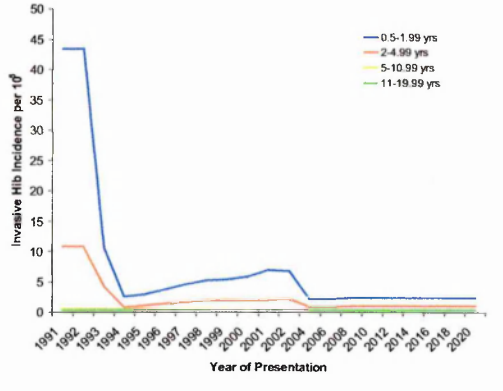


Table 7.2: Model-predicted Hib incidence rate per 100,000 in 2010 in children under 5 years of age given a range of interventions

Intervention	Boost response 100 μg/ml	Boost response 50 μg/ml	Boost response 15 μg/ml
No catchup or boost	2.52	2.52	2.88
Booster dose of vaccine given from 1993 onwards			
Boost 12 months	0.61	0.81	1.34
Boost 15 months	0.62	0.83	1.32
Boost 18 months	0.65	0.87	1.42
Boost 4 years	0.79	1.09	1.51
Catchup campaign initiated mid-2003, various levels of coverage			
20% catchup only	2.35	2.54	2.89
40% catchup only	2.42	2.57	2.90
60% catchup only	2.51	2.60	2.92
80% catchup only	2.63	2.65	2.94
Catchup campaign initiated mid-2003, accompanied by introduction of fourth dose			
20% catchup/boost 12 months	0.59	0.69	0.97
20% catchup/boost 18 months	0.63	0.85	1.41
60% catchup/boost 12 months	0.58	0.68	0.97
60% catchup/boost 18 months	0.67	0.86	1.41

Table 7.3: Model-predicted Hib incidence rate per 100,000 in 2020 (equilibrium) in children under 5 years of age given a range of interventions

Intervention	Boost response 100 μg/ml	Boost response 50 μg/ml	Boost response 15 μg/ml
No catchup or boost	2.30	2.52	2.88
Booster dose of vaccine given from 1993 onwards			
Boost 12 months	0.61	0.81	1.34
Boost 15 months	0.63	0.83	1.32
Boost 18 months	0.66	0.87	1.42
Boost 4 years	0.79	1.09	1.51
Catchup campaign initiated mid-2003, various levels of coverage			
20% catchup only	2.30	2.52	2.88
40% catchup only	2.30	2.52	2.88
60% catchup only	2.31	2.52	2.88
80% catchup only	2.31	2.52	2.88
Catchup campaign initiated mid-2003, accompanied by introduction of fourth dose			
20% catchup/boost 12 months	0.57	0.68	0.98
20% catchup/boost 18 months	0.65	0.87	1.42
60% catchup/boost 12 months	0.57	0.68	0.97
60% catchup/boost 18 months	0.66	0.87	1.42

Figure 7.1a: Invasive Hib disease incidence in children aged 0.5-1.99 years

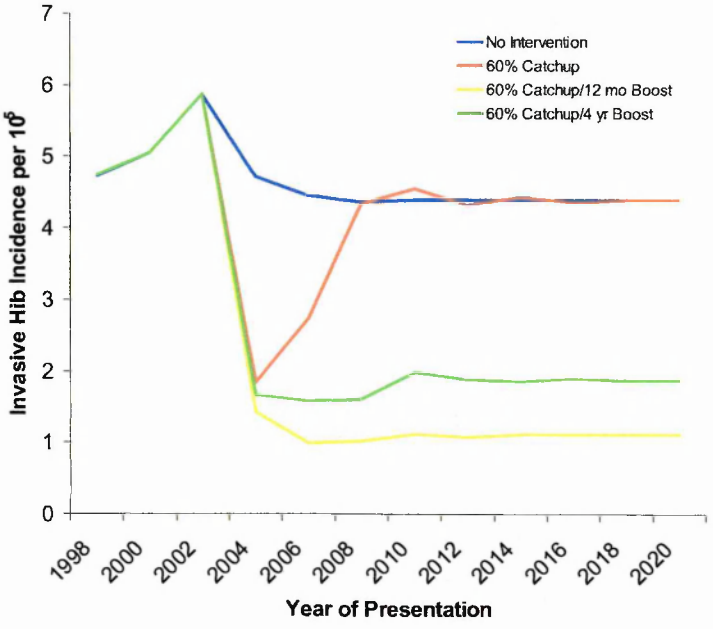
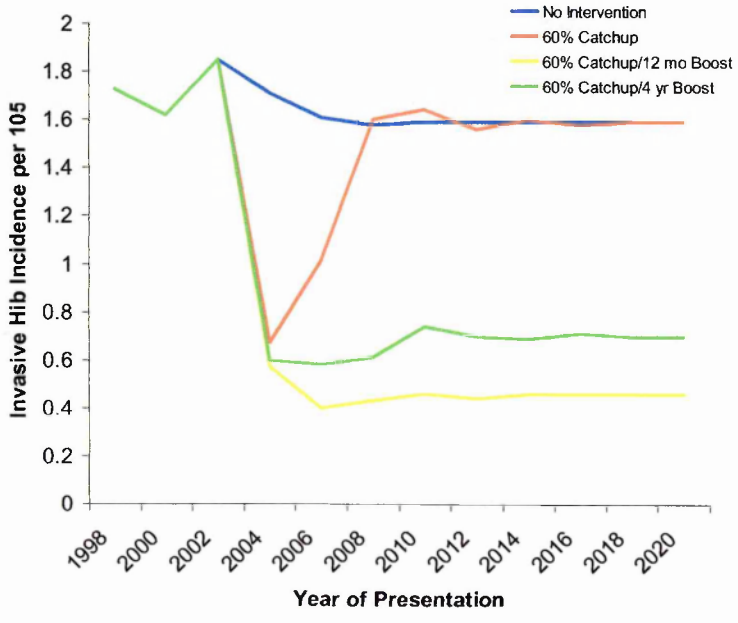


Figure 7.1b: Invasive Hib disease incidence in children aged 2-4.99 years



7.6 Summary of results of the model simulations

While it is recognised that the simulations above cannot provide true population estimates of future Hib incidence in the UK, they do allow us to draw broad conclusions about the likely relative impact of alternative interventions. Overall messages derived from this exercise, summarised in Tables 7.2 and 7.3 and Figure 7.1 were as follows:

(i) The catch-up immunisation campaign that accompanied Hib vaccine introduction was associated with a greater decrease in invasive disease incidence in the first few years of the campaign than could be expected using the routine infant schedule alone. Incidence rates of Hib disease were rising to an equilibrium point before the use of poorly immunogenic vaccines in 2000/2001.

(ii) In the absence of any specific intervention, the increase in disease associated with use of poorly immunogenic vaccines may have resolved by 2005-2006. The equilibrium incidence was closely related to the duration of protection associated with natural boosting.

(iii) The use of a catch-up immunisation campaign alone as a means of disease control produced a short-term reduction in disease incidence. This was a transient effect, however, with an associated rebound in cases within 7 years. The magnitude of this rebound increased with coverage achieved during the campaign. Following resolution of this transient behaviour, the equilibrium incidence of disease was identical to that observed without any intervention, thus conferring no long-term benefit.

(iv) In order to achieve maximum impact, routine booster immunisation was best administered between 12 and 18 months of age. Boosting at 4 years of age was too late to include the period of peak susceptibility to disease.

(v) The greatest impact of a routine booster superimposed on the 2003 catch-up campaign was observed when the fourth dose was given at 12 rather than 18 months. This combination was associated with a greater reduction in disease than if routine immunisation had been administered all along, without the catch-up. The magnitude of this effect was closely linked to the duration of protection associated with boosting.

7.7 Conclusions

The model outputs strongly suggest that, in order to obtain maximum benefit from the 2003 catch-up campaign, a routine fourth dose of Hib vaccine should be scheduled as close to 12 months of age as possible. The equilibrium incidence of disease in the absence of such a dose is estimated to be between 3 and 5 times higher than if the booster is given. The cost-effectiveness of such an intervention is likely to be a significant factor in determining whether or not it is introduced.

Chapter 8 – Ecological aspects of Hib virulence

8.0 Introduction

Prior to the use of widespread Hib vaccination, high rates of colonisation were noted in household contacts of Hib cases. Further, children with older siblings were at greater risk of invasive Hib disease. These observations led Ounsted to hypothesise that repeated passage of the organism through multiple carriers resulted in evolution of strains with heightened virulence potential (Ounsted C 1950). Subsequent reports of institutions and day care centres with a very high density of carriers, but no cases of invasive disease, made this hypothesis less credible, although the effect could be restricted to family settings (Turk DC 1963) (Murphy TV et al 1985).

In considering reasons for the recent increase in Hib disease incidence in the UK, some discussion of the possibility that immunisation has selected for more virulent Hib strains in the population is required. An evolutionary framework for consideration of such concepts is introduced. The specific question addressed in this chapter is whether changes in medical treatment of invasive Hib infections over the last century may have impacted on the life history of the organism, and thereby altered the evolutionarily stable level of virulence of circulating strains.

8.1 'Trade-off' models and evolutionarily stable levels of virulence

A number of theories have been proposed to explain the evolution of pathogen virulence in human hosts (Williams GC et al 1991) (Ebert D 1999). These may be most meaningfully examined in the context of specific infectious diseases, based on an understanding of the pathogenesis and the relevance of virulence to transmission in each

case. For organisms that have recently crossed species boundaries, virulence properties in the new host may be an unfortunate 'coincidence', not due to any characteristics that would be selected for in the original host reservoir. Unique manifestations may relate as much to the interaction of the new host's immune system with the organism as properties of the pathogen (Weiss RA 2002). For many diseases such as Hib however, humans are the unique host, and in this instance an alternative explanation for the expression of virulence must be sought.

Direct benefit models propose that the nature of the symptoms produced by the pathogen actually facilitates its transmission to the next host. Oft-quoted examples are diarrhoea induced by enteric viruses, favouring faecal-oral spread, and coughing as a feature of lower respiratory tract infection promoting aerosol spread. Invasive bacterial disease is frequently cited as a counter to such theory, as 'invasion' by definition involves the spread of an organism into a relatively inaccessible site such as the bloodstream or CSF from where transmission is unlikely to occur (Frank SA 1996) (Lipsitch M et al 1997a).

A 'trade-off' between the potential costs and benefits of virulence has been hypothesised. Characteristics of a pathogen or its disease manifestations that favour transmission may also incur cost, by placing the primary host at risk of morbidity and mortality. If the host dies, the duration of the infectious period is shortened, and there is no net benefit to the pathogen. This can be seen by considering the basic reproductive rate (R_0), the number of secondary cases produced by the introduction of one infectious individual into a fully susceptible population. The infected host remains so until he or she clears the pathogen (ρ) or dies, due to natural causes (μ) or invasive disease (α). In

a population where all are born susceptible at birth rate (B) and die at rate (μ), the R_0 of an organism with transmissibility (β) will be:

$$R_0 = \frac{\beta B}{\mu(\rho + \alpha + \mu)}$$

For R_0 to remain constant or increase, the cost of a shorter infectious period (because of faster death (α) or quicker clearance (ρ)) must be at least offset by higher infectiousness (β). Such models predict the evolution of an intermediate stable level of virulence (Frank SA 1996) (Anderson RM et al 1991). The classic biological example given to demonstrate this theory is that of the selection for avirulent strains of myxoma virus in European rabbits in Australia after it was deliberately introduced as a biological control strategy. Strains that induced high fatality rates diminished in prevalence in the population, and less virulent strains began to predominate within a matter of a few years (Levin S et al 1981, Fenner F 1983).

The survival of an organism in the population is not only based on its ability to be transmitted, however. Considerations of the trade-offs involved in fitness have been further subdivided into within-host and between-host components (Frank SA 1996). A complex range of adaptive strategies may benefit within-host survival, including tropism for specific tissues that may expand the ecological niche available (Bull JJ 1994). Within host competition depends on the relatedness of strains, and should increase in the setting of superinfection with unrelated organisms as each pathogen struggles to compete for the available niche. For closely related organisms, kin selection (Frank SA 1996) or collective action (Brown SP et al 2002) theories predict

that evolution to reduced virulence may occur. This may particularly be the case in infections where the pathogen depends not only on ready made host products for its survival, but also on substances produced by the interaction of pathogen with host, as in some viral infections (Brown SP et al 2002).

Models studying competition between unrelated strains, predict the 'tragedy of the commons' phenomenon, where 'rapacious pathogens gain a disproportionate share of host resources by rapid exploitation' (Frank SA 1996). Models of superinfection have been developed to explore the likely effect of competition on the virulence and diversity of bacterial strains encountered in human populations. Where strains can simultaneously co-infect the host, the relationship between transmission and virulence within individual strains has a marked influence on the conclusions drawn by such models. If all strains are equally transmissible, a wide range of isolates persists in the population, with the more virulent strains being less prevalent. Where the two are linked in a 'trade-off' relationship, a smaller number of strains tend to persist, of higher intrinsic virulence (May RM et al 1995). If a dominance hierarchy is assumed, where more virulent organisms replace less virulent ones, again fewer hosts are infected with strains of higher virulence, but the number of strains present in the population increases as the average virulence rises (May RM et al 1994).

The most severe end of this spectrum is the 'short sighted' evolution theory where a single 'selfish' clone may pursue short-term advantage to its ultimate cost. Such a theory has been proposed to explain progression to invasive disease by normally avirulent encapsulated organisms such as Hib (Levin BR et al 1994). Here, within-host competition results in dominance of a single virulent clone of bacteria that are capable

of invading into the bloodstream or CSF, but are not necessarily more likely to be dispersed to subsequent hosts. Based on this, Frank cautions that 'one should not mistake rare, extreme cases of virulence for the distribution of virulent effects in the population, in spite of the attention that naturally focuses on these extreme cases' (Frank SA 1996).

The importance of host immunity in shaping virulence must also be considered. The ability to evade host immune pressure is clearly going to confer survival advantage on individual parasite strains (Bull JJ 1994). Bacteria have evolved many mechanisms by which they may evade host defences, including molecules which improve attachment to host surfaces, enzymes which destroy proteins involved in the immune response and capsules which mask immunogenic surface antigens (Gotschlich EC 1983). Some have suggested that the effect of host immunity on growth rates may actually be responsible for the maintenance of virulence (Antia R et al 1994). Enhanced clearance resulting from immune mechanisms reduces the duration of the infectious period leading to selection for more transmissible pathogens that, according to the argument, harbour increased potential to cause morbidity and mortality.

Evidence for the impact of host immunity on pathogen selection is seen in the divergent population structures of different serogroups of *Neisseria meningitidis* (Gupta S et al 2001). The immune response to group A meningococcus is dominated by antibodies to the capsular polysaccharide, which is strongly immunogenic and highly conserved. Type A strains therefore compete purely on the basis of fitness within the host, as all are equally susceptible to immune attack. In contrast, the serogroup B capsular polysaccharide is a weak immunogen. As a result, host antibodies are predominantly

directed against a range of OMPs (including PorA) on the surface of the pathogen. Much less antigenic diversity is exhibited in the PorA proteins of serogroup A than B. It has been hypothesised that the diversity of group B strains is predictable because of the range of epitopes under selection pressure, resulting in the production of a series of 'small island' populations of the organism with non-overlapping antigenic variants (Gupta S et al 2001).

Widespread immunisation as a disease control strategy may thus be expected to have an effect on pathogen selection. Where the vaccine acts to reduce the potential reservoir of susceptible hosts, relatedness between strains should increase, favouring reduced virulence. Imperfect vaccines control pathogen growth rates while not eliminating them from the population, potentially selecting for increased virulence (Gandon S et al 2000, 2001). Proof of such theories would be provided by clinical evidence of worsening disease severity in the unvaccinated proportion of the population (Gandon S et al 2001). For pathogens with complex population structure and multiple serotypes, effects may be more unpredictable and will depend critically on vaccine design (Lipsitch M 1997b).

8.2 Population structure of Hib, changes in clonal distribution over time

The highly clonal population structure of Hib has been discussed at length in Section 1.2 of Chapter 1. Globally, 9 clones are responsible for the vast majority of invasive disease due to this pathogen (Musser JM et al 1990). Within a given geographic region, only one or two clones tend to predominate. Quite marked variation occurs in the dominant clones between populations however, even those that are closely related geographically and historically such as Canada and the US, or different states of the US. One explanation of this may be that Hib has a relatively slow dispersal velocity (Musser

JM et al 1990). Another may be the immunodominance of the serotype b capsule, resulting in limited genetic variation as is observed in group A meningococcus (Gupta S et al 2001). Supportive of this notion is the marked genetic diversity of nt Hi (Section 1.2) (Cody AJ et al 2003) which, like group B meningococcus, lacks an immunodominant epitope.

Changes in predominant clones within populations have been described over time. In the United States, the frequency of the clone ET-1/OMP 1H increased dramatically between 1939-1954 and 1977-1980 (Barenkamp SJ et al 1983) (Musser JM et al 1985).

Following vaccine introduction in Finland, a marked decrease was observed in the frequency of isolation of 3 of 4 common phenotypic subtypes of Hib, with a slower decrease in the 1-II-9 strain. It is of interest to note that this strain was predominant in the most densely populated region of Finland before vaccine introduction and maintained this geographical distribution. As such, opportunities for transmission may have been greater than for other strains, highlighting the ongoing importance of environmental factors to pathogen survival (van Alphen L et al 1992). A recent study of Hib strains in Alaska demonstrated variation in dominant biotypes between the early 1980s and the late 1990s, corresponding with the introduction of a national Hib immunisation programme. This change in the functionally characterised behaviour of isolates was not associated with a distinguishable shift in the genetic makeup of strains as defined by surface expressed OMP subtype (Lucher LA et al 2002).

Variation in the gene region encoding the type b capsule has been characterised. Using strains taken from cases of invasive disease in vaccine trials in the US and Finland, the

distribution of genotypes causing disease in vaccinees (72) was compared with those observed in unvaccinated controls (143). No difference in the distribution of ribotype patterns was observed, nor was there any difference in the proportions of individuals infected with isolates which were genotypically type b, but lacking capsular expression (b- strains) (Falla TJ et al 1995).

8.3 Relationship between genotype and phenotype

In attempting to interpret the above observations, it must be remembered that no consistent association has been drawn between clones or subclones of Hib and pathogenicity, making the significance of either any change or no change equally uncertain. Most clones are equally represented in collections drawn from carriers and individuals experiencing invasive disease in large population based studies. Rare exceptions are the association of ET21.8, otherwise described as OMP subtype 1c, with meningitis in Finland (Musser JM et al 1990) (Takala AK et al 1987) and the low representation of OMP subtype 13L amongst invasive isolates compared with its carriage prevalence (Hampton CM et al 1983). Based on the observation of outbreaks of Hib in children in day care centres, Barenkamp et al suggested that the OMP 1H subtype might be more pathogenic than other strains. Carriage studies performed in three nurseries where more than one case of invasive disease had been reported revealed widespread transmission of the disease causing strain from the index case for all subtypes, but a greater association of OMP 1H with secondary cases of serious infection (Barenkamp SJ et al 1981).

It therefore remains unclear what markers should be studied in an attempt to relate genotype with phenotype under selection pressure. Current techniques may detect

changes in clonal dominance, but may otherwise be blunt tools to describe changes of functional relevance to pathogenicity. 'Contingency loci' within the Hi genome have been associated with phase variable expression of characteristics that may heighten virulence by conferring increased adaptability to a range of host environments (Section 1.4 of Chapter 1) (Bayliss CD et al 2001). What would be of interest are genes that control the speed with which such new variants may be produced. Deletions in the *dam* gene of *N meningitidis* have been associated with an increased capsule phase variation rate in one laboratory, corresponding with a higher observed frequency in isolates taken from individuals with invasive disease than asymptomatic carriers. Such a finding has not been confirmed in subsequent studies, however (Bayliss CD et al 2001).

Examination of the impact of immunisation on the clinical course of Hib disease in the UK does not suggest that there has been any overall increase in the severity of disease presentations due to the organism over the past ten years. As outlined in detail in Section 2.4.1, the only epidemiological shift over the period has been an increase in the number of presentations with bacteraemia, which may well reflect changing clinical practice in the management of the febrile child. No increase in case fatality has been observed, with no differential between vaccinated and unvaccinated cases. Information on sequelae of Hib infections is lacking, and may be an area for further study. In short, while it is important to continue examining disease trends for evidence of changing Hib virulence, no evidence of such an effect of immunisation has been observed to date.

8.4 Potential impact of antimicrobial therapy on life history of Hib

Changes in both the population structure (Musser JM et al 1985) and incidence of invasive Hib infections were reported from the US over the second half of the 20th

century. Centres in the US and Canada noted a marked increase in the number of presentations with bacterial meningitis in children due to Hib, compared with other pathogens, between 1950 and 1970 (Koch R et al 1961, Gossage JD 1964, Haggerty RJ et al 1964, Michaels RH 1971, Finland M et al 1997). This time course corresponded with the use of life-saving antimicrobial therapy. Could antibiotic use and enhanced survival have conferred fitness benefit on disease associated ET1/OMP-1H clones, which also became more prevalent over this time period (Musser JM et al 1985)?

Evolutionary trade-off models, as discussed in Section 8.1, make much of the impact of death as a consequence of virulence on a pathogen's life cycle. Prior to the advent of antibiotics, the case fatality rate associated with bacterial meningitis caused by Hib was in the order of 80-100%, making the assumption of a trade-off justified. From the 1940s onwards, however, combination therapy with sulphonamides and penicillins, often coupled with chloramphenicol or streptomycin, reduced this death rate to below 10%. If a trade-off between transmissibility and virulence did exist for Hib, what might have been the consequence of removing this 'cost' of virulence from the equation?

Children convalescing from Hib disease following antimicrobial therapy revealed ongoing carriage of the organism for up to a year. Thus, the duration over which the organism might be transmitted was no longer shortened by progression to invasive disease. A higher than expected carriage rate of Hib was further seen in their immediate family members (Michaels RH et al 1977, Li KI et al 1986) and day care contacts (Ward JI et al 1978, Murphy TV et al 1983a). This elevated carriage prevalence was also observed in secondary contacts, namely the parents and siblings of asymptomatic Hib carriers who had associated with an index case of invasive disease in a day care

nursery setting. Of interest, colonisation rates in these secondary contacts were higher when the primary contact carried a Hib strain identical to the index case as opposed to an unrelated strain, but this difference did not achieve significance (Table 8.0) (Li KI et al 1986).

Table 8.0: Carriage of Hib in Secondary Household Contacts of an Index Case in Day-Care

Characteristics of Household Primary Contact	Colonisation Rates			
	Number of households	Total positive swabs	Parents	Siblings
Household of colonised day-care classmate (all)	21	10/48 (21%)	5/36 (14%)	5/12 (42%)
Primary contact carries OMP subtype identical with index patient	14	8/32 (25%)	4/23 (17%)	4/9 (44%)
Primary contact carries OMP subtype different from index patient	7	2/16 (13%)	1/13 (8%)	1/3 (33%)

Associated with this evidence of exposure was an estimated risk of secondary disease in household members under 5 years of age as high as 4%, 500 times higher than the population risk (Ward JI et al 1979). The likelihood of a secondary case occurring in a day nursery contact was harder to estimate, but ranged from 0-4% in four large prospective studies in the United States (Fleming DW et al 1985, Makintubee S et al 1987, Osterholm MT et al 1987, Murphy TV et al 1987).

Such observations may be consistent with the hypothesis that strains of Hib that cause invasive disease are more efficiently transmitted than other strains. No logical direct benefit of the disease state itself can be proposed, and indeed individuals asymptomatically carrying invasive disease causing strains transmit just as efficiently. The way in which these two attributes are linked is unclear, but may be through growth

rates or other characteristics that are coded for by genes in linkage disequilibrium. As described above, there is no clear evidence for distinct Hib clones being involved in the pathogenesis of invasive disease, but current molecular methods are blunt tools, and the genetic basis of virulence imperfectly understood.

Subsequent recognition of the risk of secondary cases, as well as the risk of recurrent infection in the index case due to ongoing colonisation, led to the recommendation of rifampicin therapy to eradicate carriage in Hib cases and contacts (Cartwright KAV et al 1991). Such therapy had a 98% short-term efficacy at eliminating the carrier state (Glode MP et al 1985), and documented benefit in the prevention of further cases in both the home and nursery settings for children under 4 years of age (Band JD et al 1984). The introduction of such an intervention on a large scale might be expected to once again increase selection pressure on the organism by removing disease-causing strains, reversing the fitness benefit resulting from enhanced host survival.

A simple strain competition model was developed in order to simulate the divergent effects of:

(i) Antimicrobial therapy to treat invasive disease alone

(ii) Antimicrobial therapy to halt transmission of disease causing strains

8.5 Strain competition model to simulate impact of antimicrobial therapy on fitness

Suppose two Hib strains, or clones, coexist in the population. Strain 1 is less infectious than strain 2, but also less likely to invade and cause disease. Based on the absence of demonstration of Hib co-infection in carriage studies (Section 1.2), it is assumed that individuals are only infected with one strain at any given time. Following infection with one strain, some measure of natural immunity ensues, which confers equal cross

protection against both strains. This assumption is based on the observation that the immunodominant antigen in Hib is the capsular polysaccharide, which is highly conserved (Section 1.9). The corresponding host immune response to this antigen is also highly conserved, with a limited number of germline encoded heavy and light chain regions dominating the antibody response across a number of distinct populations (Section 1.11) (Lucas AH et al 1999). Prior to the advent of antibiotic therapy, invasive disease due to either strain resulted in an 80% case fatality rate. The model describing the behaviour of the two strains is shown in Figure 8.0.

Susceptibles are born into a fully susceptible state, S at birth rate B, and die at rate μ. Strain 1 is initially circulating in the population, and strain 2 is introduced at time t=250. Hosts become infected with either strain at a rate dependent on the intrinsic transmissibility of each (β_1 and β_2) and the number of individuals in the population infected with either strain. These may be asymptomatic carriers (I_1 and I_2) or be experiencing invasive disease (D_1 and D_2). No increased infectiousness is conferred by bacterial invasion. Individuals recover from the carrier state at rate ρ_1 and ρ_2 respectively to a relatively resistant state, R. From this state, there is a 50% reduced risk of infection with either strain. The alternative to recovery is progression to invasive disease (at rate δ_1 or δ_2). Diseased individuals have an increased risk of death (α_1 and α_2) in addition to the background mortality rate, μ. Those who survive recover at rates ρ_{D1} and ρ_{D2} to the carrier state, and from there to the resistant class. With use of Rifampicin therapy, the situation changes and diseased cases recover directly to the resistant class, at rates ρ_{D1}' and ρ_{D2}' (denoted by dashed line in the model). The partial protection provided against infection by previous exposure is only temporary, as it wanes at rate ω.

The series of ordinary differential equations describing this model is as follows:

$$dS/dt = B - [\beta_1(I_1 + D_1) + \beta_2(I_2 + D_2) + \mu]S + \omega R$$

$$dI_1/dt = \beta_1(I_1 + D_1)S + 0.5\beta_1(I_1 + D_1)R - (\rho_1 + \delta_1 + \mu)I_1$$

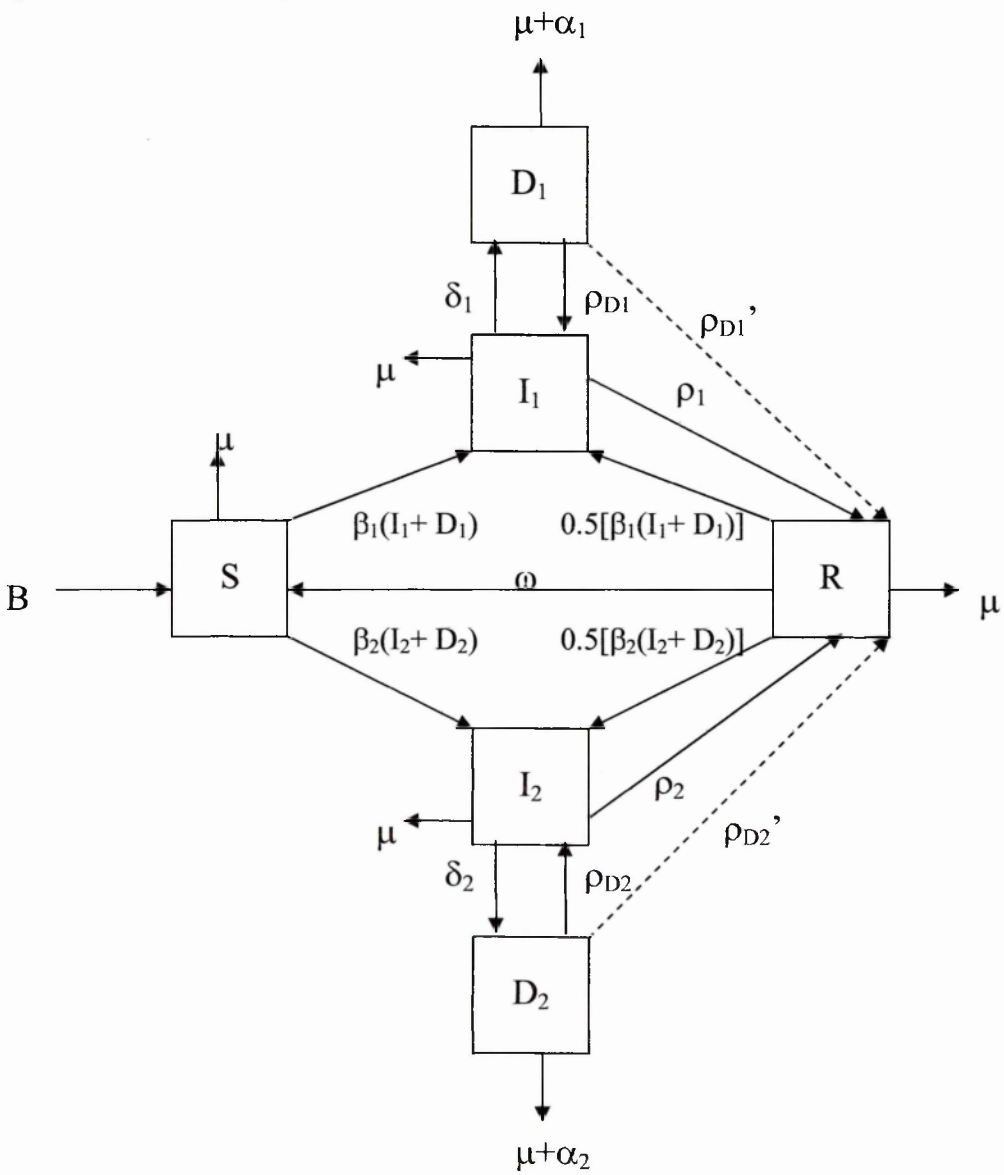
$$dI_2/dt = \beta_2(I_2 + D_2)S + 0.5\beta_2(I_2 + D_2)R - (\rho_2 + \delta_2 + \mu)I_2$$

$$dD_1/dt = \delta_1 I_1 - (\rho_{D1} + \alpha_1 + \mu)D_1$$

$$dD_2/dt = \delta_2 I_2 - (\rho_{D2} + \alpha_2 + \mu)D_2$$

$$dR/dt = \rho_1 I_1 + \rho_{D1} D_1 + \rho_2 I_2 + \rho_{D2} D_2 - [0.5\beta_1(I_1 + D_1) + 0.5\beta_2(I_2 + D_2) + \omega + \mu]R$$

Figure 8.0: Strain competition model



A total population size of 500,000 was assumed. The annual rate of births balanced deaths, keeping total population size stable.

$B = 10{,}000$ $\mu = 0.02$

Asymptomatic infection persisted for 2 months, with invasive disease lasting 1 month.

$\rho_1 = \rho_2 = 6$ $\rho_{D1} = \rho_{D2} = \rho_{D1}' = \rho_{D2}' = 12$

Partial protection against infections from the resistant state waned to give an average duration of protection of 2 years.

$\omega = 0.5$

Strain 1 was less virulent than Strain 2: 1.5% of infections due to the former strain progressed to invasive disease, and 3% of the latter. Calculated values for δ were as follows:

$\delta_1/(\delta_1 + \rho_1 + \mu) = 0.015$ $\delta_2/(\delta_2 + \rho_2 + \mu) = 0.03$

$\delta_1 = 0.09$ $\delta_2 = 0.19$

The case fatality rate, given progression to invasive disease, was assumed to be the same for both strains, falling from 80% to 10% with availability of antibiotics.

Pre-Antibiotics:	Antibiotic era:
$\alpha/(\alpha + \rho_D + \mu) = 0.8$	$\alpha/(\alpha + \rho_D + \mu) = 0.1$
$\alpha_1 = \alpha_2 = 48.08$	$\alpha_1 = \alpha_2 = 1.34$

The total force of infection, λ, under initial conditions was assumed to be 0.3. Under this assumption the average age at first infection would be approximately 3 years. Equilibrium values for each of the state variables using the above parameter set were calculated using matrix algebra in Excel. The value of the transmission coefficient β_1 could then be derived, given that $\lambda = \beta_1(I_1 + D_1)$. β_2 was set to be slightly higher than β_1, thus correlating transmissibility with virulence.

$\beta_1 = 1.58 \times 10^{-5}$ $\beta_2 = 1.59 \times 10^{-5}$

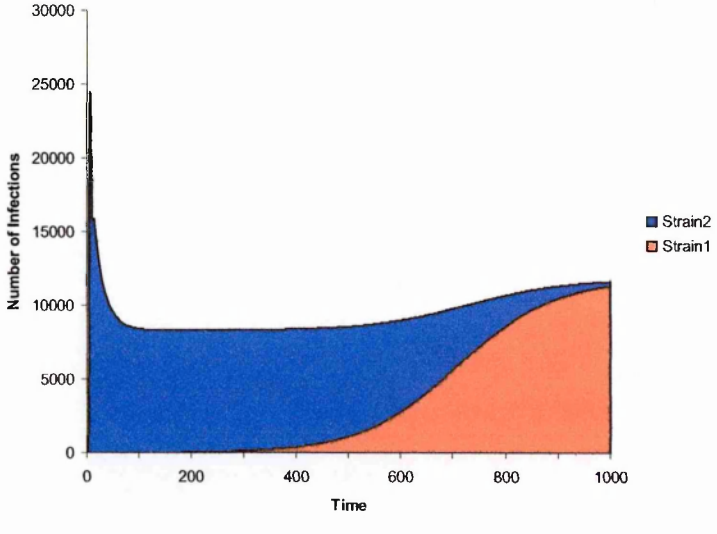
The basic reproductive rate (R_0) for each pathogen was calculated using next generation matrices (Diekmann O et al 1990). Within this model, there are two types of infectious individuals, carriers and diseased, potentially producing the same two kinds of secondary infections. Consequently, R_0 is as follows for each strain:

$$R_{01} = \frac{\beta_1 B}{\mu(\rho_1 + \delta_1 + \mu)} + \frac{\delta_1 \beta_1 B}{\mu(\rho_1 + \delta_1 + \mu)(\rho_{D1} + \alpha_1 + \mu)}$$

$$R_{02} = \frac{\beta_2 B}{\mu(\rho_2 + \delta_2 + \mu)} + \frac{\delta_2 \beta_2 B}{\mu(\rho_2 + \delta_2 + \mu)(\rho_{D2} + \alpha_2 + \mu)}$$

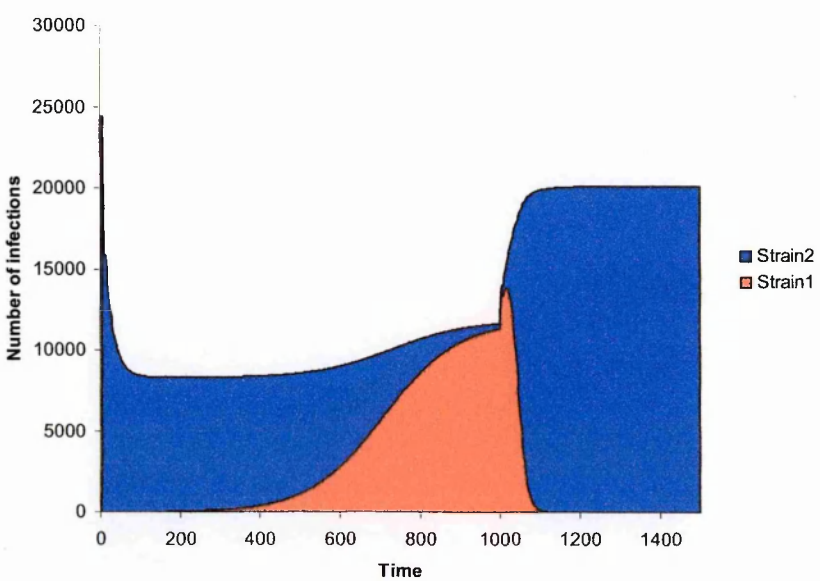
Prior to introduction of antibiotics, $R_{01} = 1.293769$ and $R_{02} = 1.2875$. The dominance of Strain 1 can be seen when it is introduced into a population at time t=50 initially infected with Strain 2 in Figure 8.1.

Figure 8.1: Balance of Strains from the competition model, Strain 1 introduced at time t=50



With introduction of successful antibiotic therapy, reducing the case fatality rate from 80% to 10%, the dominance of strains is reversed: $R_{01} = 1.300986$ and $R_{02} = 1.301841$. The subsequent effect on the prevalence of each strain (colonisation and invasive disease episodes) in the population is seen in the simulation below, where the intervention is introduced at time t = 1000. As Strain 2 is far more likely to cause invasive disease than Strain 1, the overall number of disease cases as a proportion of these infections at equilibrium is increased. At time t = 1000, there are 19 invasive disease cases due to Strain 1 and one due to Strain 2. By time t = 2000, there is no disease attributable to Strain 1, but 285 cases of Strain 2. The relationship of the two strains over time is illustrated in Figure 8.2.

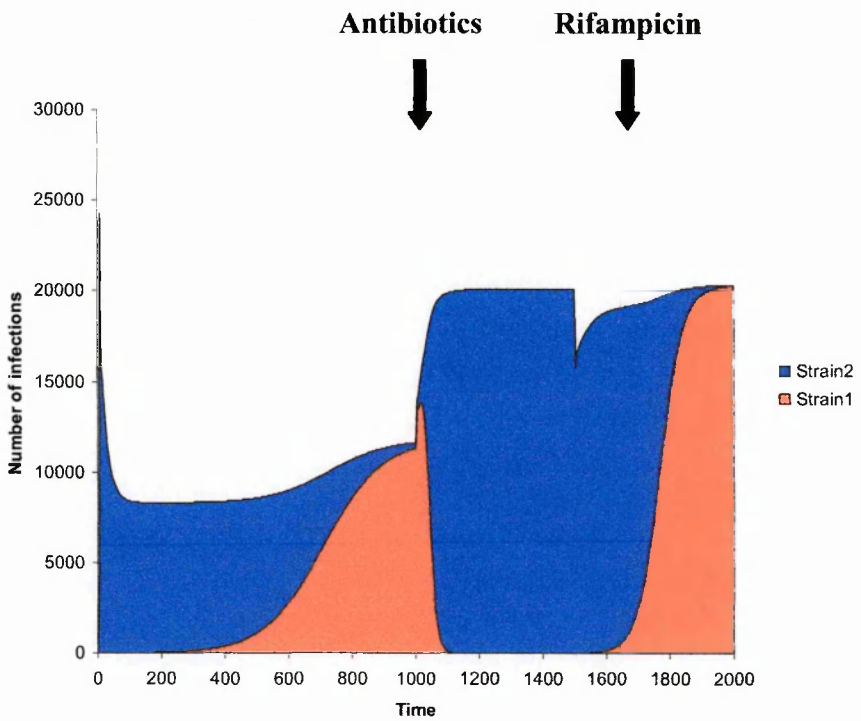
Figure 8.2: Balance of strains from the competition model, with introduction of antibiotic therapy at time t=1000 (all infections for each strain)



Thus, it can be seen that the more transmissible strain, with its associated higher risk of causing invasive disease, is benefited when the 'cost' of virulence is removed. Far from being removed from circulation by the death of the host, the organism is able to continue to reproduce in the population. The use of chemoprophylaxis to eradicate

disease causing organisms places a new 'cost' on more invasive strains, resulting in a reversal of this effect (Figure 8.3).

Figure 8.3: Effect of Introducing Rifampicin to Eradicate Carriage in Individuals Recovering from Invasive Disease (at time t=1500)



8.6 Conclusion

Widespread immunisation against Hib has the theoretical potential to exert evolutionary pressure on the organism, resulting in altered intrinsic virulence. Clinical indicators of such change might include differences in the spectrum or severity of disease presentations, particularly in unvaccinated children. In addition, evidence of increased invasive potential in older age groups in the face of measurable natural immunity may imply emergence of immune 'escape' mutants. No such impact on Hib epidemiology has been observed in Britain (Section 2.4.1). The only consistent association with the

increased disease rates observed in the UK has been a reduction in the prevalence of detectable serum antibody titres at both ends of the age spectrum (Section 3.3).

Genetic studies of Hib have been unable to define markers consistently associated with phenotype. The same strains appear equally likely to cause both asymptomatic colonisation and serious infection. No shift in the population structure of the organism has been observed following the introduction of Hib vaccination in several countries studied. Interpretation of the consequences of this finding for phenotype is difficult, given the limitations of current molecular tools.

Evolutionary 'trade-off' models provide a framework within which to explore the consequences of intervention on the life history of pathogens. Such models assume that the benefit of virulence, arbitrarily defined as heightened transmissibility, is balanced by its cost, which is death of the host. The introduction of antibiotics to treat Hib infections largely removed death from the equation, and could thus be predicted to have a major impact on the evolutionarily stable level of virulence.

An increase in the observed incidence of Hib meningitis in the US was reported in the second half of the 20th century, coinciding with availability of antimicrobial therapy. Over the same period a new clone became predominant in the population. A simple strain competition model was used to show the way in which a reduction in case fatality may switch the dominance of two circulating strains. Use of Rifampicin to block ongoing transmission of disease causing isolates reversed this trend, by again introducing a 'cost' of invasion to the pathogen.

Chapter 9 – Summation

9.0 Summary of findings

Conjugate vaccines have been remarkably effective at reducing transmission of Hib in countries where they have been introduced for widespread use (Section 1.15). In addition, they are able to directly protect even very young infants against invasive disease. (Section 1.15) The exquisite susceptibility of children under the age of 2 years to serious Hib infections has been attributed to immune hyporesponsiveness to the Hib capsular polysaccharide, PRP (Section 1.9). The efficacy of conjugate vaccines in this age group correlates with their ability to induce levels of anticapsular antibody deemed to correlate with clinical protection.

What are the key components of vaccine induced protection against Hib? Based on this understanding, how many doses of Hib vaccine are required to protect children over the first five years of life? Early success of the UK's Hib immunisation programme, which relies purely on an accelerated primary infant schedule without a booster dose, led some to assert with confidence that immunologic memory would provide clinical protection even after anticapsular antibodies had waned, making a routine fourth dose of vaccine unnecessary (Booy R et al 1997) (Heath PT et al 2000a). Recent experience in the UK suggests that such may not be the case (Section 2.4.1).

Lower serum antibody titres to the Hib capsular polysaccharide PRP in the population have been associated with greater disease risk in the following studies:

- Antibody titres wane rapidly following primary infant immunisation – a corresponding decline in vaccine efficacy was observed when the first two years following vaccination were compared with subsequent years (Section 3.1.1).
- In contrast, high levels of immunity were achieved and maintained over the first 5 years of life following catch-up immunisation, as employed in the UK in 1992/3 (Section 2.6.2). Corresponding estimates of efficacy were far higher than those calculated following the infant primary course (Section 3.1.1).
- Introduction of a poorly immunogenic DTaP-Hib combination vaccine in 2000/2001 was temporally associated with higher rates of invasive disease and vaccine failure (Section 2.4.2), a reduced duration of protection following immunisation (Section 2.4.2) and declining vaccine efficacy (Section 3.1.1). Inferior protection at an individual level was confirmed by a case-control study comparing vaccines received by children presenting with invasive Hib infection with those administered to healthy controls (Section 3.1.2). Children who had received two or three doses of the primary immunisation series as DTaP-Hib were significantly more likely to present with breakthrough infection than those who had been given three doses of another Hib vaccine.
- In keeping with the notion that lower antibody responses are associated with inferior protection, a significantly increased risk of Hib disease has been noted in infants born before 37 weeks' gestation (Section 3.2.3). Such children demonstrate reduced post-primary and post-booster antibody concentrations (Section 3.2.2).

An additional impact of widespread Hib immunisation has been a reduction in circulation of the organism in the community (Section 2.5.1). While providing excellent indirect protection against disease by reducing opportunities for acquisition, such an effect may be

anticipated to have adverse consequences for maintenance of immunity in the medium to long term for an infection such as Hib, where resistance is not life long.

- A decline in the prevalence of protective Hib antibody titres was observed in older unimmunised cohorts within only a few years of introduction of Hib vaccine (Section 2.4.3). A subsequent rise in invasive disease incidence has been observed in these age groups, particularly among adults in their 30s and 60s, who are likely to be in close household contact with infants and young children.
- In a similar fashion, less effective maintenance of post immunisation immunity may be expected to occur in children immunised in more recent years than early in the national Hib campaign, when ongoing carriage could still be documented in nursery settings (Section 2.5.1).
- A national study of social and environmental factors associated with ongoing Hib infection identified nursery attendance and household exposure to cigarette smoke as variables reducing the risk of invasive Hib disease in vaccinated children (Section 3.2.3). This was in direct contrast to observations before vaccine availability, as both of these exposures have been linked with increased Hib carriage in the past (Section 1.6). Nursery attendance in particular was a significant risk factor for invasive disease in unimmunised children (Section 1.8). A possible explanation for these surprising findings may be that a consistent level of background exposure to the organism assists in the maintenance of vaccine induced immunity.

British children are dependent on immunologic memory for protection once antibodies have waned to low or undetectable levels. However, many children who experience invasive Hib disease demonstrate evidence of previous priming for memory responses

(Section 3.2.2) that has been insufficient to prevent bacterial invasion. If one considers the estimate of vaccine efficacy obtained two years following primary infant immunisation to be a proxy measure of the protection afforded by memory, this may be as low as 27% (Section 3.1.1). Seroepidemiologic studies have revealed an increase in the proportion of individuals without measurable antibody in many age groups in the UK over the past 10 years (Section 2.6.2). Greater susceptibility to bacterial invasion at the point of exposure to Hib may conceivably result in a higher incidence of Hib disease for a given background carriage prevalence.

Mathematical models of Hib infection and immunisation were developed in order to allow study of the effects of interruption of transmission on levels of protective immunity in the population. Further, they provided a framework within which to investigate key assumptions regarding the mechanism of vaccine protection. Conjugate vaccines were assumed to have two main effects based on an understanding of their immunologic properties as TD antigens:

(i) Generation of antibody levels deemed protective following immunisation in infancy

(ii) Induction of immunologic memory, manifest as 'booster' antibody responses following re-exposure to the pathogen or subsequent vaccination

Vaccine induced immunity was otherwise assumed to act in a similar way to natural immunity. Features of interventions unique to the UK, such as an initial catch-up immunisation campaign and the use of less immunogenic vaccines over a two-year period, were able to be incorporated.

The high coverage achieved for the catch-up immunisation campaign had a rapid and substantive impact on reducing all infections, but also resulted in large transient effects. The second of these rebounds in circulation coincided with the use of poorly immunogenic vaccines in the model and was amplified in consequence (Section 6.1). The relatively short time scale seen for these transient effects resulted from the intrinsic properties of the system (Section 6.2). Strong damping was observed, due to both the relatively long duration of infection and the reversion of resistant individuals to the susceptible state (Section 6.2.2).

Model outputs describing the proportion of the population resistant to Hib acquisition, and those without detectable immunity, showed good correlation with the observed seroprevalence data over a ten year period (Figure 6.14b)) (Figure 6.17). The effect on carriage prevalence in children of nursery age was also within the 95% confidence limits of population measures (Figure 6.14a)). Strongly assortative mixing patterns, with a significant contribution of an older sibling to infection risk in young infants, achieved the observed relative balance of invasive disease in children of different age groups (Section 6.3).

Having achieved a reasonable fit to measures of infection and immunity, the relative protection afforded against acquisition (Section 5.4) (Section 6.5) and progression to invasive disease (Section 5.6) (Section 6.5) could also be calculated against the UK incidence data. In order to recapitulate both excellent initial control of disease and a late resurgence in infections, the parameters describing efficacy at both of these stages could only be in the order of 25-35% (Section 6.5). This estimate of the direct protection

afforded by memory was very similar to that calculated at a population level using the screening method (Section 3.1.1).

It is interesting that a resurgence in Hib infections in vaccinated children in Alaska occurred within 5 years of the introduction of a conjugate vaccine programme. Associated features at the time included very high rates of carriage in older children, and the use of HbOC in infants (Section 1.16). This vaccine produces much lower post-primary immunisation antibody titres than the formerly used PRP-OMP. In a setting where the median age at presentation with Hib infection was substantially younger than that in the UK, this opening of a susceptible six-month window was sufficient to result in a substantial increase in infections, perhaps fuelled by transient behaviour. The importance of maintaining high anticapsular antibody titres in order to protect infants from infection is reinforced by this additional population experience.

The possibility that the intrinsic virulence of Hib may be increasing under selective pressure induced by immunisation was considered. There are no clinical indicators from the epidemiology of ongoing Hib infections to suggest any worsening of disease presentations or increase in case fatality in either vaccinated or unvaccinated individuals to suggest that this is the case (Section 2.4.1) (Section 8.3). The absence of any clear genetic markers of virulence for Hib makes monitoring of such a possibility at the molecular level difficult (Section 8.3). No shifts in clonal population structure have been observed following vaccine introduction in communities where this question has been addressed (Section 8.2).

9.1 Immunologic implications of the UK Hib experience

If increasing dependence on immunologic memory is to be blamed for the UK's increase in invasive Hib disease, we must further pursue the additional question: why was the protective efficacy of memory so much lower than anticipated?

9.1.1 Should memory be expected to protect at all?

Rapidly rising antibody titres have been observed in both vaccinated (McVernon J et al 2003b) and unvaccinated (Anderson P et al 2000) children presenting with invasive Hib infections. Such responses were consistent with previous priming, and yet these children were not protected. Why was memory insufficient to prevent disease in these cases?

Memory is only one component of the immune repertoire in the face of bacterial invasion (Lucas AH et al 2001). As our understanding of immunology improves, the associations between genotype and immune responsiveness to a range of diseases become more complex (Kwiatkowski D 2000). The critical role of individual susceptibility in determining the likelihood of bacterial invasion is strongly implied by the fact that, prior to vaccine availability, 5% of children under 5 years were colonised with Hib at any given time, yet only 30 per 100,000 would go on to experience invasive disease. Why were some children vulnerable and not others? Factors other than memory are likely to play an important role (Lucas AH et al 2001).

Some believe that protective immunity and memory are distinctly separate entities. It has been postulated that memory exists primarily to allow mothers to pass on antibodies to their unborn infants (Zinkernagel RM 2000) and should not be relied upon for protection.

Such a view is at one extreme end of the spectrum, but perhaps provides an appropriate note of caution.

9.1.2 Do memory responses continue to provide the same qualitative protection, regardless of time since last exposure to antigen?

A debate rages in immunology over whether or not maintenance of pools of memory cells requires ongoing exposure to the initiating antigen. Many assert that the process is antigen independent (Maruyama M et al 2000), with separate populations of memory lymphocytes and long-lived plasma cells persisting for many years without the need for additional stimulation (Slifka MK et al 1995). Alternatively, polyclonal activation of the long-lived memory cell pool has been invoked as an explanation for the continual production of new populations of antibody forming cells (Bernasconi NL et al 2002). While measurable antibody responses have been noted in adoptive transfer experiments of memory lymphocytes in mice, their functional capacity may suffer in the absence of both antigen and T cells (Zinkernagel RM 2002).

The alternative view proposes that ongoing specific stimulation of memory pools of cells is needed (Zinkernagel RM 2002). In the case of immunisation, ongoing antigenic exposure may be endogenous, due to persistence of vaccine in immune complexes on dendritic cells (Zinkernagel RM 2000). In the UK, three doses of Hib conjugate vaccine are administered, at 2, 3 and 4 months of age. For lifelong immunity, 3 doses of tetanus toxoid are insufficient, with 5 doses required to ensure persistence of protection (Gray D 2001). Are infants in the UK receiving an insufficient quantity of antigen, in the absence of a fourth dose?

Such a question becomes more important when one considers that opportunities for exogenous Hib exposure have been markedly reduced by widespread immunisation (Adegbola RA et al 1998, Barbour ML et al 1995, Fernandez J et al 2000, Takala AK et al 1991). Cross-reactive capsular antigens on organisms such as E coli K100 were believed to be important in priming for Hib responses in infancy. They do not, however, consistently bind to antibodies produced in response to PRP vaccine, or following invasive Hib infection (Insel RA et al 1982). As such, these organisms would seem unlikely to boost immunity to Hib that has been primarily induced by vaccination. Antibody titres in adults were seen to wane within only a few years of vaccine introduction, in keeping with the anticipated decrease in Hib exposure (Section 2.4.3). How long should we expect functional memory responses to persist in such a situation?

The mathematical models of Hib infection developed (Chapters 4, 5, 6, 7) assumed that boost responses would continue to have the same qualitative and quantitative attributes, regardless of the time since immunisation or last natural exposure. Were this assumption to prove incorrect, one may predict a substantial reduction in long-term population protection against Hib resulting from immunisation.

9.1.3 Are there reasons why memory induced by vaccination may be inferior to that generated by natural exposure to the intact organism?

In the natural protective response to encapsulated bacteria, T cells that recognise bacterial proteins presumably help the B cells that recognise the capsular polysaccharide (Lucas AH et al 1999). Conjugate vaccines employ carrier proteins such as diphtheria and tetanus toxoids to elicit T cell help, rather than peptides specific to Hib bacteria. T cells involved

in the initial response to such conjugates are specific for the carrier protein (Perez-Melgosa M et al 2001).

Following Hib conjugate priming, boost responses are observed with administration of plain PRP. Tenfold higher titres can be elicited by re-immunisation with conjugate vaccine, however (Goldblatt D et al 1999). This discrepancy would imply that while primed B cells are capable of responding to re-exposure to the polysaccharide antigen, the assistance of specific T cell help improves the magnitude of antibody production with a subsequent stimulus. While not necessarily required for natural immunity, T cells may provide a valuable adjunct in the protection of large populations (McVernon J et al 2004c).

A reduction in the circulating Hib pool has removed opportunities both for boosting B cell polysaccharide specific immunity, and also T cell immunity to exposed surface proteins. T cells may be important both in the maintenance and effector phases of the specific immune response to Hib. Thus, a reduction in transmission, so beneficial to herd immunity, may be a double-edged sword with negative as well as positive consequences for protection (McVernon J et al 2004c).

9.2 Wider implications of the UK Hib experience

The most obvious additional case to which this analysis is relevant is the meningococcal serogroup C (Men C) conjugate vaccine, also administered in the UK at 2, 3 and 4 months of age without a booster dose. This vaccine, introduced routinely as a primary infant course in late 1999, is required to provide protection well into the second decade of life when there is a peak in morbidity and mortality due to serogroup C meningococcus (Balmer P et al 2002). The implication of the present hypothesis is that if vaccine failures occur (i.e.

infection in vaccinated children), we can expect to find them late after vaccination, when antibody levels have waned and the lack of helper T cells begins to be felt (McVernon J et al 2004c).

There are also significant implications for the debate surrounding possible introduction of the more recently licensed pneumococcal conjugate vaccines. Proponents in favour of wide scale implementation in the UK quote vaccine efficacy of 97.4% (McIntosh EDG et al 2002), a figure derived from experience of a four dose course administered at 2, 4, 6 and 12-15 months (Black S et al 2000). Whether or not a three dose accelerated primary schedule would deliver a similar level of protection must substantially influence considerations of the cost-effectiveness of such a proposal.

In contrast to Hib, susceptibility to invasive disease caused by the pneumococcus is evident both during the first five years of life, and in adults aged 65 and over (Sleeman K et al 2001). Introduction of routine infant pneumococcal conjugate vaccination in the United States (US) has been associated with a significant reduction in invasive pneumococcal disease in all age groups over 20 years (Whitney CG et al 2003). Only time will tell whether a resurgence of infections will be observed in the medium term, similar to the UK's experience with Hib. Should such an increase occur, the greater underlying susceptibility of the elderly to this pathogen might be associated with a more marked elevation in the number of adult cases.

9.3 Where to from here? Regaining control of invasive Hib infections in the UK

The addition of a fourth dose of Hib vaccine as was given to children aged between 6 months and 4 years in the national 'catch-up' campaign outlined in the Chief Medical Officer's letter of the 14th of February 2003 (http://www.doh.gov.uk/cmo/index.htm) is expected to provide protective levels of antibody to a cohort of children throughout the risk period for Hib disease. Whether a booster dose will be routinely required once less immunogenic vaccines have been withdrawn from the infant immunisation course requires further study.

Investigation of the models of Hib infection and immunisation developed suggested that the recent increase in invasive Hib disease should resolve with the withdrawal of DTaP-Hib vaccine, returning to a stable equilibrium within a matter of years (Section 7.4). Exploration of the possible impact of a range of interventions indicated that this equilibrium point would not be shifted by a one off catch-up immunisation campaign, such as that employed in the UK in the second half of 2003 (Section 7.5). In addition, the transients associated with such a campaign would be likely to result in a further brief increase in infections in seven years' time.

Implementation of a routine fourth dose of vaccine would be predicted to result in a lower equilibrium incidence of Hib disease in children less than 5 years of age (Section 7.3, Section 7.5). Maximum benefit would be obtained if this vaccine was administered as close to 12 months of age as possible, due to the exquisite age dependency of susceptibility to bacterial invasion (Section 7.5). Had such a dose been incorporated in the routine schedule from the outset, it is unlikely that the use of DTaP-Hib vaccines would have

resulted in an increase in serious Hib infections, regardless of ongoing transmission in the wider community (Section 7.3).

Data from the second half of 2003 have confirmed a reduction in the number of Hib reports from the UK, compared with the same period in the preceding year. Coverage figures from the catch-up campaign are still being collated. The HPA is conducting further studies to investigate the anticipated duration of protection of a routine additional dose of Hib vaccine administered at different ages. The effect of any such intervention, should it be instituted, will continue to be monitored using the population surveillance mechanisms already in place, which have allowed early detection and a timely response to the current problem.

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