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SURVEILLANCE NETWORK FOR INVASIVE *HAEMOPHILUS INFLUENZAE* IN EUROPE - 1999 & 2000

Final report

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SUMMARY

Introduction

Decision No. 2119/98/EC for setting up a network for the epidemiological surveillance and control of communicable diseases in the Community stated as a priority "Diseases prevented by vaccination". *H. influenzae* infection comes within this priority. Using the framework already established in a BIOMED II Hib surveillance project (1996-1999), a DG SANCO surveillance network project for invasive *H.influenzae* disease was established in all 15 EU countries and 3 non-EU countries (2000-2001).

Aims

- To improve the epidemiological information on invasive *Haemophilus influenzae* disease within the European Union.
- To improve the laboratory capacity to accurately characterise the isolates *of H. influenzae*.
- To form a focus for wider collaboration with non European Union countries and candidate European Union countries.

Methods

Agreed usage of a minimum dataset and a standardised case definition for *H. influenzae* has enabled valid comparisons to be made of the disease epidemiology within Europe, and hence assist the monitoring of epidemiological changes. Information collected on the surveillance systems and the vaccination programme(s) in use by each participant country has also aided interpretation of the epidemiological analyses.

Improvements in the laboratory capacity within the EU to accurately identify *H. influenzae* have been achieved through gaining information on systems in use by participants, by running a laboratory workshop for new members to the network, and by undertaking an External Quality Assurance Scheme (EQAS) with the participant reference laboratories. The EQAS helped identify any existing problems in correctly serotyping *H. influenzae* isolates, and enabled corrections/assistance in laboratory methods to be made, hence improving comparability of data between countries. The laboratory workshop run for new members ensures standardised methods are being used, adding further to correct identification of isolates within the EU.

Results and Conclusions

Prior to introduction of Hib vaccination programmes the epidemiology of invasive Hib disease differed between the EU countries, with incidence rates in children under five varying between 12 and 60 per 100,000. The only country in this collaboration with no vaccination programme has demonstrated incidence rates in the same range (17/100,000). All EU countries now have national immunisation programmes, and therefore the incidence in children under five years, the age group with the highest incidence pre-vaccine, is now very low. Countries are at different stages of vaccine implementation, have different vaccines and schedules and have achieved different levels of coverage. Despite all of these considerations, the incidence of Hib infection in the EU is much lower than in the pre-vaccine era (between 0 and 3.0 per 100,000).

Surveillance systems varied slightly amongst the participating countries. As most countries include all invasive Hib disease in children under fifteen years, comparison of rates in under fives and under fifteens can be made. Differences may be explained by many factors, including different methods of surveillance and completeness of ascertainment. One of the most important factors is the microbiological practice in relation to the diagnosis of Hib disease. This practice can impact on the establishment of disease burden and on comparisons between countries. The importance of continued improvement of laboratory techniques and laboratory-based surveillance cannot be over-emphasised.

Although the incidence has fallen in countries using vaccine, the clinical presentation of Hib disease has not changed. Meningitis remains the predominant diagnosis, causing over 66% of disease in under two year olds, with epiglottitis being the second most common diagnosis in pre-school children. Pneumonia and bacteraemia are more common presentations in adults. Apparent differences between countries may be explained by different age distributions of cases and the small numbers of cases.

Amongst children under five in the EU countries, the highest incidence rates in 1999 were in Ireland and Italy. Rates were also high in Australia. These countries were amongst those reporting the lowest coverage in the previous project (funded under DGXII). An increase in coverage in Australia (personal communication Peter McIntyre) is likely to have explained the reduction observed in that country in 2000.

In 2000, the highest incidence was observed in the UK, who experienced almost a doubling of the number of cases and this increase has continued during 2001 (personal communication, Dr Mary Slack). One of the major differences between the UK and Ireland and the remaining EU countries is the absence of a booster (third or fourth dose) in the second year of life. Although Ireland has a comparatively high rate, no obvious trend has been observed in 2000. Rates between years in each participant country vary due to small numbers, but the increase observed in the UK and Germany, two of the largest populations under surveillance, was responsible for an overall increase in incidence in the EU in 2000. The increase in Germany, however, was of a similar magnitude to that observed in the UK, despite the use of a booster vaccination in that country.

Changes in vaccination programmes have occurred over time and may be responsible for changes in incidence observed. It is unlikely that the increase observed so far can be explained by changes in the vaccine, and if a change in vaccine was implicated, we would expect to see increases in many EU countries. The importance of continued observation over the whole of the EU is therefore essential to ensure that such changes can be detected at the earliest possible stage.

Rates of non-b capsulated *H. influenzae* infection are low and no evidence of serotype replacement has been observed despite many years of vaccination in many of the EU countries. Rates of non-capsulate infection are now similar to those for type b and emphasises the importance of ensuring accurate identification of the organism in a national reference centre. The low rates observed in some countries, probably reflects the low proportion of strains that are referred and highlights the potential for improving ascertainment of such cases.

1. INTRODUCTION

Decision No. 2119/98/EC for setting up a network for the epidemiological surveillance and control of communicable diseases in the Community stated as a priority "Diseases prevented by vaccination". *H. influenzae* infection comes within this priority.

The BIOMED II Hib surveillance project in 9 EU countries and 2 non EU countries (1996-99) was established to describe the epidemiology of invasive *Haemophilus influenzae* and describe the risk factors associated with vaccine failure using different vaccines and schedules. Using the framework already established in the above project, a DG SANCO surveillance network project for invasive *H.influenzae* disease was established in all 15 EU countries and 5 non-EU countries (2000-2001) to improve epidemiological information and laboratory capacity to characterise isolates of these two invasive bacterial infections.

Aims

To improve the epidemiological information on invasive *Haemophilus influenzae* disease within the European Union.
To improve the laboratory capacity to accurately characterise the isolates *of H. influenzae*.
To evaluate the impact of vaccination with conjugate vaccines on the epidemiology of *H. influenzae*.
To compare the impact of vaccination with conjugate vaccines produced by different manufacturers and according to different schedules.
To form a focus for wider collaboration with non European Union countries and candidate European Union countries.

A European Union network for the surveillance of *Haemophilus influenzae* is important for the following aspects within the Community : pooling of case data; pooling of vaccine failure data; rapid alert of changes in the epidemiology of infection strains; setting standards. The collection of data at European level will be available to member states to inform policy development within each country. This may therefore contribute to the harmonisation of European Hib vaccine policy and schedules.

As *Haemophilus influenzae* disease in a vaccinated community is rare, this project allows pooling of such data to increase the power of any epidemiological analysis. Hib vaccine has been demonstrated to reduce nasopharyngeal carriage of Hib and it has been postulated that one consequence of reduced exposure to this organism could be the early waning of vaccine induced immunity. In addition, the potential emergence of non-vaccine preventable strains of *H. influenzae* has been suggested. European wide analysis should be able to detect an increase in cases of Hib in older children or adults, or an increase in the incidence of non-b *Haemophilus influenzae* at an earlier stage than analysis of a single country's data. In addition, by pooling data from all countries, the populations under surveillance will become sufficient to provide more precise estimates of vaccine efficacy and will be composed of a wide variety of ethnic groups. These estimates based on pooled data may be able to assess the potential decline in vaccine efficacy with age or in certain groups.

Hib disease in vaccinated children is extremely rare. Pooling of data on vaccine failures at European level is the only reliable means of describing potential risk factors specific to certain social situations or ethnic groups, and collection of data at a European level will also increase the ethnic and social diversity of the population under surveillance.
An established network is needed for the rapid dissemination of changes in the epidemiology of an infection which may have public health significance. In addition, it will facilitate the rapid exchange of information on imported strains *of H. influenzae* infections.

This project, which has included all 15 EU countries, Iceland and Norway, and 3 countries from outside the EU, will be able to set standards for the epidemiological surveillance of *H. influenzae* and for methods used in reference laboratories. Countries will be able to learn from models of good practice in other member states and these standards can also be applied in other countries, especially candidate EU and non-EU countries. In addition, establishment of this network may facilitate early dissemination of advances in therapy and in public health control measures and lead to the harmonisation of guidance on meningococcal disease. This project will also provide a model and focus for future research and public health collaborations, for example the evaluation of other new vaccines such as conjugate pneumococcal vaccines.

In this report a summary is given of the up-to-date epidemiological information gained by collecting and analysing *H. influenzae* disease case data from the network participants for years 1999 and 2000, and displays the ability of this now established system to monitor changes in the epidemiology of the disease.

Finally, this project will provide substantial and up-to-date epidemiological information from which *H. influenzae* vaccination policy can be developed within individual countries introducing vaccination programmes, and help the development of guidance on prevention and control of meningococcal infection. It may also facilitate the eventual harmonisation of vaccine schedules in the European Union.

2. METHODS

Questionnaires on the surveillance system(s) and the laboratory diagnostic methods were sent to all new participant countries, and updates gained from countries already established as members of the network. The information from both these questionnaires is important for correct interpretation of the data which was gained from each individual country. A vaccination programme questionnaire was also administered to each new participating country, and updates obtained, where necessary, from existing members.

A minimum data set was received from the majority of countries for both 1999 and 2000. The minimum data set includes age, sex, date of onset, method of confirmation, site of identification, grouping, typing and subtyping results (as appropriate) (Refer Appendix 2). These datasets were in most cases electronically transferred to PHLS Communicable Disease Surveillance Centre, where they were entered onto the main Access database. In some instances paper listings of cases were received. The standardised case definitions developed as part of the DG XII project are used, and where surveillance is performed using other definitions, datasets are re-coded to provide comparable data for all participating countries.

Descriptive epidemiology is analysed using standard statistical packages on the minimum data set. Analysis of age-specific incidence rates, temporal trends and diversity of *H. influenzae* infections are compared. In countries with vaccination programmes, coverage data will also be requested and comparison of rates of infection in both vaccinated and unvaccinated cohorts will be interpreted in conjunction with coverage, schedule and vaccine used, since implementation and method of introduction

An External Quality Assurance Scheme (EQAS) was performed in collaboration with the reference laboratories from all participating countries. This was led by Dr Mary Slack of the PHLS *Haemophilus* Reference Unit. Standard micro reagents were used. A panel of well characterised strains of each of *H. influenzae* were freeze-dried, and a selection sent to each national or regional reference laboratory. These laboratories characterised the strains according to their routine practice and returned the results to the co-ordinating laboratory. The results of the testing were compared with known identity of the organism and returned to each centre. Aggregate results were anonymised and shared amongst the project participants, and discussion of problems with identification given.

A *H. influenzae* laboratory workshop was held in Oxford early 2001 for microbiologists from new participant countries. Skills in specific laboratory methods were shown and then practiced by all those attending. A presentation was given on the epidemiology of the disease within the participant countries, also.

A central resource was provided in the UK to genotype strains from countries with established Hib vaccination programmes. Protocols for PCR genotyping were supplied by the PHLS Haemophilus Reference Unit, Oxford, UK, for laboratories wishing to establish their own system for genotyping strains of *H.influenzae.* For those countries not wishing to establish or use this method the Oxford laboratory offered to genotype any strains isolated from vaccine failure cases.

A presentation on the epidemiology of *H. influenzae* type b in the EU countries was also shared with the EC-funded EUVAC project at a meeting in Rome in mid 2001. The EUVAC project is led by Denmark.

Dissemination of results from the surveillance of invasive *H. influenzae* disease in the EU occurred through annual reports to the network participants of the epidemiological data analyses, and presentation of results at meetings and scientific conferences. Feedback reports were given to microbiologist network participants on the External Quality Assurance Scheme (EQAS).

3. RESULTS

The original participant countries in the network (1996-1998) were Finland, Germany, Greece, Germany, Ireland, Italy, Netherlands, Spain(Valencia), Sweden, England & Wales, Israel and Australia. New members to the surveillance network are Austria, Belgium, Denmark, France, Iceland, Luxembourg, Norway, Portugal, and the Czech Republic. Of the new members, Belgium and Luxembourg provided no disaggregated data for either 1999 or 2000, Austria was only able to provide aggregated data for 1999, and Sweden was only able to provide disaggregated data for 1999.

3.1 Questionnaire surveys

3.1.1 Surveillance systems

3.1.1.1 Objectives

For countries with vaccination programmes, the objective of the surveillance was to monitor the impact of vaccination by universal case ascertainment of invasive Hib disease. In Portugal the additional objective was to monitor antibiotic resistance in cases of *Haemophilus influenzae* infection. In the Czech Republic the principal objective was the assesssment of the disease burden to inform decisions about the introduction of Hib vaccine.

3.1.1.2 Case definitions

The case definition used in each country, except Denmark and Finland, included all cases of invasive Hib disease with isolates from a sterile site. Denmark limited surveillance to meningitis. In Finland the case definition of 'invasive infection' for *H. influenzae* disease consists of blood and CSF isolations, but not isolations from other usually sterile sites.

Antigenic diagnosis was included in the case definitions used by Australia, Finland and Italy (although some other countries reported such cases to the European data set). Australia was the only country to accept a clinical, non-microbiological diagnosis of epiglottitis (although these were not included in the study data set).

Data on other serotypes was also collected in Finland, Germany, Greece, Ireland, Italy, Sweden, the Netherlands, and the UK (England & Wales).

3.1.1.3 Population under surveillance

All participant countries, except Germany, Greece, Sweden, Israel and Austria, had a surveillance system across all ages. In Austria, Sweden, Germany, and Israel, cases were only reported in the paediatric population. In Germany the paediatric reporting involves cases up to the age of 10 years, but laboratory reporting includes older cases. As cases between 10 and 14 years are unusual and to allow comparisons, surveillance in Germany has been assumed to cover the whole population under fifteen. In Greece (Attiki) surveillance was limited to to paediatric population (under 15 years) in a single region and in Italy enhanced surveillance was performed in seven regions.

3.1.2 Hib vaccination programmes

The details of the type of vaccines used and the immunisation schedules in the ongoing programmes are given below (Table 1). There is considerable variation between countries in the vaccines and schedules used. As well as countries concurrently using more than one vaccine type, the type(s) being

used by a country has changed over time with the continual emergence of new Hib vaccines from the range of manufacturers. Also, a high proportion of the Hib vaccines used are now combination vaccines; possible components being DTaP, DTwP, IPV, or Hepatitis B.

Table 1 : Type of Hib conjugate vaccine and immunisation schedule used in the study participant countries

Country/region	Type of vaccine	Combined with	Immunisation schedule
Australia	HbOC (90%) PRP-OMP (10%)		2, 4, 6, 18 months 2, 4, 12 months
Austria	2000 • Infanrix + Hib (SKB) • Infanrix-IPV+Hib (SKB) • Tetravac (Aventis Pasteur MSD) 2001 As above, plus Hexavac (DTaP-Hib-IPV-HBV)	 DTaP DTaP, IPV DTaP, IPV DTaP, IPV, HBV	 3, 4, 5 months & 2nd year of life 3, 4, 5 months & 2nd life of life 3, 4, 5 months & 2nd year of life
Belgium	*Not yet available*		
Czech Republic	No programme in 1999/2000		
Denmark	PRP-T (Act-HIB)		3, 5 12 months
Finland	HbOC		4 months (with DTP) 6 months (with IPV) 14-18 months (with MMR)
France	Hib PRP-T	DTwP, DTaP, IPV	Pentacoq • 2, 3, 4, 18 months Pentahibest • 2, 3, 4, 18 months Pentavac • 18 months Infanrix Polio Hib • 18 months

Table 1 : Type of Hib conjugate vaccine and immunisation schedule used in the study participant countries (continued)

Country/region	Type of vaccine	Combined with	Immunisation schedule
Germany			Recommended primary schedule at 2-3 months of age
	PRP-OMPC (Pocomvax)	Hep B	
	PRP-D (HIB-DT Merieux)	DT	2 doses at 6-8 wk intervals
	PRP-T (Pentavac)]	DTaP-IPV	3 doses at 4 wk intervals
	PRP-T (Infanrix-IPV+HIB)] 90%	DTaP-IPV	3 doses at 4 wk intervals
	PRP-T (Infanrix+HIB)]	DTaP	3 doses at 4 wk intervals
	PRP-D (HIB-Vaccinol)		3 doses at 4 wk intervals
	PRP-D (HIB-Merrieux)		2 doses at 4-6 wk intervals
	PRP-T (Act-HIB)		2 doses at 4-8 wk intervals
Greece	PRP-T		2, 4, 6, 15-18 months
	HbOC		2, 4, 6, 15-18 months
	1999 PRP-T	DTaP, IPV	2, 4, 6, 18 months
	HbOC		
Iceland	PRP-D ProHIBit		3, 4, 6, 14 months
	Jan 2000 onwards PRP-T (Pentavac)	DTaP, IPV	3, 5, 12 months
Ireland	Pre August 2001		
	PRP-T (ACTHib or HibTITRE(60%), Hiberix(30%)		2, 4, 6 months
	Post August 2001		
	PRP-T (Pentavac) (100%)	DTaP, IPV	2, 4, 6 months
Israel	1994-1997		
	PRP-OMP (90%)		2, 4, 12 months
	HbOC/PRP-T		
	Jul 1997 onwards PRP-T		2, 4, 6, 12 months
	1999 PRP-T	DTwP	
	HboC	DTwP	

Table 1 : Type of Hib conjugate vaccine and immunisation schedule used in the study participant countries (continued)

Country/region	Type of vaccine	Combined with	Immunisation schedule
Italy	PRP-T HbOC for a few months in 1996 Apr 1999 onwards PRP-T	 DTaP, IPV, Hep B	<6 months (3 doses + booster) >12 months (1 dose) 6-12 months (2 doses + booster) >12 months (1 dose) 3, 5, 11-12 months
Luxembourg	*Not yet available*		
Netherlands	PRP-T 1999 PRP-T	DTP, IPV (in other limb)	3, 4, 5, 11 months 2, 3, 4, 11 months
Norway	PRP-T (100%)	DTaP, IPV	3, 5, 12 months
Portugal	*Not yet available*		
Spain (Valencia)	PRP-T (30%) HBOC (70%)		As recommended by the manufacturers (4 doses < 12 months, 1 dose >12 months)
Sweden	PRP-T		3, 5, 12 months
United Kingdom	HBOC PRP-T DTwP/PRP-T since 1996 (some DTaP used in 2000)	DTwP	2, 3, 4 months 2, 3, 4 months 2, 3, 4 months

3.1.2 Laboratory questionnaire

The questionnaire on laboratory methods was returned by nineteen countries: Australia (Melbourne and Sydney) Austria, Belgium,Czech Republic, Denmark, Finland, France, Greece, Iceland, Ireland, Israel, Italy, Luxembourg, Netherlands, Portugal, Spain, Sweden and the UK).

3.1.2.1 Laboratory Hib identification and reference facilities.

All countries , except Greece, had reference laboratory facilities for *Haemophilus influenzae.* All countries had primary identification in over 80% of laboratories, except Greece where such facilities were available in only 50-80% of laboratories.The proportion of laboratories that referred isolates of *Haemophilus influenzae* to the reference laboratory ranged from <20% in Belgium and Sweden to 100% in Australia, Austria, Finland, Iceland, Ireland, Luxembourg and UK.

In most countries those hospital laboratories that could identify *H.influenzae* would normally test all specimens from cases of suspected bacterial meningitis and all blood cultures for *H.influenzae.* In Luxembourg 80-100% of hospital laboratories had facilities to identify *H.influenzae* and would only test specimens from cases of meningitis. In this country, only CSF isolates of *H.influenzae* were referred to the reference laboratories. In Sweden only strains from possible vaccine failure cases would be referred to the reference laboratory. Some hospital laboratories would look for *H.influenzae* in sterile site specimens such as joint aspirates.

3.1.2.2 Specimen transport, receipt and storage

All reference laboratories subcultured the strains immediately on receipt and either tested them immediately or in batches. All the media used to transport the strains to the reference laboratory and to subculture the strains were able to sustain the growth of *H. influenzae.* All but one of the laboratories (Greece) could store the strains long term at –80C.

3.1.2.3 Identification Methods, serotyping and genotyping

There were some minor differences in the identification methods used. The type of blood used in the preparation of blood and chocolate agar plates varied. In most countries that responded to the question either horse or sheep blood was used. In two countries (Austria and Greece) human blood was used. This is not to be recommended since human blood may be inhibitory to the growth of *H.influenzae.*

With the exception of Austria, all reference laboratories serotyped strains of *H.influenzae.* Austria stated that they planned to introduce serotyping shortly. The main difference in the identification methods used by the laboratories related to the ability to genotypically confirm vaccine failures as *H.influenzae* type b. Genotyping facilities were available in Australia, France,Italy and UK. The strains from vaccine failure cases in Ireland are sent to the PHLS Haemophilus Reference Unit in Oxford (UK).

3.1.2.4 Access to laboratory methods

A central resource was provided in the UK to genotype strains from countries with established Hib vaccination programmes. Protocols for PCR genotyping were supplied by the PHLS Haemophilus Reference Unit, Oxford, UK, for laboratories wishing to establish their own system for genotyping strains of *H.influenzae.* For those countries not wishing to establish or use this method the Oxford laboratory offered to genotype any strains isolated from vaccine failure cases.

3.2 Laboratory External Quality Assurance (EQA) for Haemophilus influenzae

Of the 18 centres participating in the quality assurance scheme , 1 laboratory failed to perform their results and 2 laboratories failed to accept the invitation to participate. A total of 13 sets of completed results were returned.

Strain 3 was *Haemophilus influenzae* type b (biotype IV), 15 centres correctly identified this strain as serotype b.
Comments : There were no problems with this strain.

Strain 4 was *Haemophilus influenzae* type c (biotype IV). 14 centres correctly identified this strain as serotype c, 1 centre identified it as a non-typable strain and 1 commented that it was polyagglutinable.
Comments : This strain gave a clear positive reaction with polyvalent and type c *Haemophilus influenzae* antisera.

Strain 7 was *Haemophilus influenzae* type f (biotype IV). 15 centres correctly identified this strain as serotype f.
Comments : There were no problems with this strain.

Strain 18 was *Haemophilus influenzae* type b (biotype I). 15 centres correctly identified this strain as serotype b. This strain was ß-lactamase positive and chloramphenicol acetyl transferase positive. It was resistant to ampicillin /amoxycillin, chloramphenicol, tetracycline and kanamycin. 9 centres performed antimicrobial susceptibility tests on the strains. All 9 centres correctly identified this strain as ß-lactamase positive and ampicillin resistant. 6 centres also reported the strain as chloramphenicol and tetracycline resistant.
Comments : There were no problems with this strain.

Strain 20 was a *Haemophilus influenzae* non-capsulated (biotype II). 15 centres correctly identified this strain as a non-capsulated strain.
Two laboratories suggested that this strain might be a ß-lactamase negative ampicillin resistant strain.
Comments : The sensitivities of this strain were checked by E-test MICs. The following results were obtained. Ampicillin (MIC 0.5 :g/ml) co-amoxyclav (MIC 4 :g/ml) cefuroxime (MIC 4 :g/ml) and cefotaxime (MIC 0l08 :g/ml). The strain is thus susceptible to ampicillin and cefotaxime and could be regarded as susceptible or of intermediate susceptibility to co-amoxyclav and cefuroxime. The strain is not a BLNAR.

Strain 23 was *Haemophilus influenzae* non-capsulated (biotype III). 11 centres correctly identified this strain. 2 centres incorrectly identified this strain as type a, 1 centre identified it as type b and 1 centre found it to be type c. **Comments :** This strain gave non-specific agglutination with more than one monospecific typing antiserum. PCR may be required to confirm the serotype.

3.3 Data on invasive *Haemophilus influenzae* infection 1999-2000

3.3.1 Overall incidence of invasive Hib disease

Data on cases in all age groups was provided by 8 European countries (Czech Republic, Finland, Ireland, Italy, Netherlands, Norway, Portugal and the UK) and by Australia, for 1999 and 2000 (Table 2). Data on meningitis in all age groups was supplied by Denmark. The crude incidence was low in the European Union countries in 1999 and 2000 (0.12 and 0.13 per 100,000 population, respectively). All these EU countries have vaccination programmes established. In 1999 and 2000 the highest incidence was seen in the Czech Republic – the only country without national vaccination programme. Of those countries with a vaccination programme, Ireland had the highest incidence rate (0.19) in 1999, and the UK had the highest (0.19) in 2000.

3.3.2 Age disribution of cases

Amongst those EU countries with surveillance in all age groups, the overall percentage of cases in children under 5 years of age was 57% and 64% in years 1999 and 2000, respectively. (Table 3) This percentage ranged widely within, and between all the reporting countries (14%-100%) over these two years. Four countries showed a decrease in the percentage of cases in the under five population, while five showed an increase, and one remained the same. However, account must be taken of the low number of cases some countries are experiencing now they have had vaccination programmes running for a substantial period of time. The Czech Republic, which did not have a vaccination programme instituted in 2000, had an age distribution similar to all the other countries in the network prior to vaccination introduction: over 75% of the cases in children under 5 years of age.

The overall percentage of cases in children under one year of age in EU countries reporting Hib cases in all age groups was 27% in 1999, and 19% in 2000. The Czech Republic showed percentages of 18 and 13 for these years, while the other countries ranged between zero and 100 percent of cases being present in the under one year age group.

3.3.3 Incidence of invasive Hib disease in childhood

Data on all cases in children under 15 years was provided by 12 European Union countries (Austria, Ireland, Finland, Germany, Greece, Iceland, Italy, Netherlands, Norway, Portugal, Sweden, UK), and by three countries outside the EU (Australia, Czech Republic, Israel). (Table 4) Denmark provided data on meningitis only. The annual incidence in the EU increased from 0.33 per 100,000 in 1999, to 0.38 per 100,000 in 2000. In 1999 the highest incidence (0.88) was seen in Austria, and in 2000 it was seen in the UK (0.68).

The incidence in the EU was higher in children under five than in those under fifteen, and increased between 1999 (0.85) and 2000 (1.06). (Table 5) The highest rate in 1999 in the under fives was in Italy (2.96). The UK had the highest rate in 2000, and rates of above one per 100,000 were observed in Ireland and Italy, also. A sharp increase was observed in both the UK and Germany between 1999 and 2000 and formed the major contribution to the overall increase.

Overall incidence in the EU for Hib meningitis in children under 5 years of age also saw an increase from 1999 (0.55) to 2000 (0.59). (Table 6) The major contributors to this increase were Germany and

the UK, with lesser additions being made by Finland, Ireland and the Netherlands. In Australia, Hib meningitis incidence in under fives displayed a marked decrease (0.92 to 0.39) over the two years. The Czech Republic, being at the pre-vaccination programme stage, has high rates for 1999 and 2000 (10 and 11 per 100,000, respectively). These rates are comparable to those seen in Greece, Ireland, Spain and the UK at a similar point in the Hib disease epidemiology.

Table 2 : Numbers of cases and crude incidence (per 100,000 population) of invasive Hib disease for all age groups, by country : 1999 & 2000

Country	Year	<1 y	1 yr	2 yrs	3 yrs	4 yrs	5-9 yrs	10-14 yrs	15+yrs	NK	Total cases	Population	Rate
Denmark*	1999	1	0	0	0	0	0	0	3	0	4	5313577	0.08
	2000	1	0	0	0	0	0	0	0	0	1	5330020	0.02
Finland	1999	2	0	0	0	0	0	1	4	0	7	5171302	0.14
	2000	1	0	0	0	1	0	0	0	0	2	5171302	0.04
Iceland	1999	0	0	0	0	0	0	0	0	0	0	269,735	0.00
	2000	0	0	0	0	0	0	0	0	0	0	269,735	0.00
Ireland	1999	1	2	0	0	0	1	0	3	0	7	3626087	0.19
	2000	1	1	0	1	0	0	0	0	0	3	3626087	0.08
Italy (enhanced)	1999	17(7)	6(3)	9(2)	2(0)	0(0)	2(0)	0(0)	8(5)	0(0)	44(17)	27,880,793	0.16
	2000	9(8)	2(0)	4(2)	1(1)	2(2)	0(0)	1(1)	2(2)	0(0)	21(16)	27,880,793	0.08
Netherlands	1999	5	0	1	1	0	1	0	4	0	12	15619082	0.08
	2000	3	3	2	0	0	0	0	7	0	15	15619082	0.10
Norway	1999	0	2	0	0	0	0	0	3	0	5	4578497	0.11
	2000	0	0	1	0	0	0	0	6	0	7	4578497	0.15
Portugal	1999	2	0	0	0	0	0	0	0	2	4	9920762	0.04
	2000	0	0	1	1	0	0	0	0	1	3	9920762	0.03
UK	1999	12	6	5	4	5	4	0	30	0	66	51911175	0.13
	2000	15	10	12	15	9	5	2	31	2	101	51911175	0.19
EU TOTAL*	**1999**	**29**	**16**	**15**	**7**	**5**	**8**	**1**	**52**	**2**	**145**	**118977433**	**0.12**
	2000	**29**	**16**	**20**	**18**	**12**	**5**	**3**	**46**	**3**	**152**	**118977433**	**0.13**
Australia	1999	10	8	2	0	0	1	1	5	0	27	18311486	0.15
	2000	6(4)	2	1(0)	1	0	3	1	0	0	14(11)	18311486	0.08
Czech Rep.	1999	17	18	16	13	14	9	0	5	0	92	10282784	0.89
	2000	14	29(27)	10	16(15)	15(13)	13	1	7	0	105(100)	10282784	0.97

* Denmark reports only meningitis and is therefore excluded from the EU totals
* Numbers in parentheses indicate cases confirmed by isolation in countries where antigen detection is included

Table 3 : Age distribution of cases of invasive Hib disease by country for 1999 and 2000

Country	Year	Under 1		1-4 years		0-4 years		5-14 years		15+ years		Total
		No	%	No	%	No	%	No	%	No	%	
Denmark*	1999	1	25%	0	0%	1	25%	0	0%	3	75%	4
	2000	1	100%	0	0%	1	100%	0	0%	0	0%	1
Finland	1999	2	29%	0	0%	2	29%	1	14%	4	57%	7
	2000	1	50%	1	50%	2	100%	0	0%	0	0%	2
Iceland	1999	0	-	0	-	0	-	0	-	0	-	0
	2000	0	-	0	-	0	-	0	-	0	-	0
Ireland	1999	1	14%	2	28%	3	43%	1	14%	3	43%	7
	2000	1	33%	2	67%	3	100%	0	0%	0	0%	3
Italy (enhanced)	1999	17	39%	17	39%	34	77%	2	5%	8	18%	44
	2000	9	43%	9	43%	18	86%	1	4.8%	2	10%	21
Netherlands	1999	5	42%	2	17%	7	58%	1	8.3%	4	33%	12
	2000	3	20%	5	33%	8	53%	0	0%	7	47%	15
Norway	1999	0	0%	2	40%	2	40%	0	0%	3	60%	5
	2000	0	0%	1	14%	1	14%	0	0%	6	86%	7
Portugal	1999	2	100%	0	0%	2	100%	0	0%	0	0%	2
	2000	0	0%	2	100	2	100%	0	0%	0	0%	2
UK	1999	12	18%	20	30%	32	48%	4	6.1%	30	45%	66
	2000	15	15%	46	46%	61	62%	7	7.0%	31	31%	99
EU TOTAL*	**1999**	**39**	**27%**	**43**	**30%**	**82**	**57%**	**9**	**6.3%**	**52**	**36%**	**143**
	2000	**29**	**19%**	**66**	**44%**	**95**	**64%**	**8**	**5.4%**	**46**	**31%**	**149**
Australia	1999	10	37%	10	37%	20	74%	2	7.4%	5	19%	27
	2000	6	43%	4	29%	10	71%	4	29%	0	0%	14
Czech Rep.	1999	17	18%	61	66%	78	85%	9	9.8%	5	5.4%	92
	2000	14	13%	70	67%	85	81%	14	13%	7	7.0%	105

* Denmark reports only meningitis and is therefore excluded from the EU totals

Table 4 : Numbers of cases and crude incidence (per 100,000 population) of invasive Hib disease in children under 15 years of age, by country : 1999 & 2000

Country	Year	<1 yr	1-4 yrs	5-9 yrs	10-14 yrs	Total cases	Population	Rate
Austria	1999					12	1356807	0.88
	2000	N/A	N/A	N/A	N/A	N/A		
Denmark*	1999	1	0	0	0	1	967643	0.10
	2000	1	0	0	0	1	981148	0.10
Ireland	1999	1	2	1	0	4	859424	0.47
	2000	1	2	0	0	3	859424	0.35
Finland	1999	2	0	0	1	3	943001	0.32
	2000	1	1	0	0	2	943001	0.21
Germany	1999	2	8	3	0	13	12897014	0.10
	2000	10	11	2	2	25	12897014	0.19
Greece	1999	1	0	0	0	1	558558	0.18
	2000	N/A	N/A	N/A	N/A	N/A		
Iceland	1999	0	0	0	0	0	64470	0.0
	2000	0	0	0	0	0	64470	0.0
Italy (enhanced)	1999	17(7)	17(5)	2(0)	0(0)	36(12)	3595194	1.00
	2000	9(8)	9(5)	0(0)	1(1)	19(14)	3595194	0.53
Netherlands	1999	5	2	1	0	8	2915911	0.27
	2000	3	5	0	0	8	2915911	0.27
Norway	1999	0	2	0	0	2	882408	0.23
	2000	0	1	0	0	1	894717	0.11
Portugal	1999	2	0	0	0	2	1744600	0.11
	2000	0	2	0	0	2	1744600	0.11
Sweden	1999	1	4	0	1	6	1654452	0.36
	2000	N/A	N/A	N/A	N/A	N/A		
UK	1999	12	20	4	0	36	10033595	0.36
	2000	15	46	5	2	68	10033595	0.68
EU TOTAL*	**1999**	**43**	**55**	**11**	**2**	**123**	**37505434**	**0.33**
	2000	**39**	**77**	**7**	**5**	**128**	**33947926**	**0.38**
Australia	1999	10(10)	10(10)	1(1)	1(1)	22(22)	3911737	0.56
	2000	6(4)	4(3)	3(3)	1(1)	14(11)	3911737	0.36
Czech Republic	1999	17(17)	61(61)	9(9)	0(0)	87(87)	1728678	5.03
	2000	14(14)	70(65)	13(13)	1(1)	98(93)	1685398	5.81
Israel	1999	3	3	0	0	6	1638400	0.37
	2000	6	3	2	0	11	1638400	0.67

* Denmark reports only meningitis and is therefore excluded from the EU totals
* Numbers in parentheses indicate cases confirmed by isolation in countries where antigen detection is included

Table 5 : Numbers of cases and crude incidence rate (per 100,000 population) in children under 5 years of age, by country : 1999 & 2000

Country	Year	<1 yr	1 yrs	2 yrs	3 yrs	4 yrs	Total cases	Population	Rate
Denmark*	1999	1	0	0	0	0	1	344685	0.29
	2000	1	0	0	0	0	1	340593	0.29
Ireland	1999	1	2	0	0	0	3	250394	1.20
	2000	1	1	0	1	0	3	250394	1.20
Finland	1999	2	0	0	0	0	2	297522	0.67
	2000	1	0	0	0	1	2	297522	0.67
Germany	1999	2	2	5	1	0	10	3947634	0.25
	2000	10	6	3	2	0	21	3947634	0.53
Greece	1999	1	0	0	0	0	1	169648	0.59
	2000	N/A	N/A	N/A	N/A	N/A	N/A		
Iceland	1999	0	0	0	0	0	0	20981	0.00
	2000	0	0	0	0	0	0	20981	0.00
Italy (enhanced)	1999	17(7)	6(3)	9(2)	2(0)	0(0)	34(12)	1147352	2.96
	2000	9(8)	2(0)	4(2)	1(1)	2(2)	18(13)	1147352	1.57
Netherlands	1999	5	0	1	1	0	7	976175	0.72
	2000	3	3	2	0	0	8	983491	0.81
Norway	1999	0	2	0	0	0	2	301963	0.66
	2000	0	0	1	0	0	1	302387	0.33
Portugal	1999	2	0	0	0	0	2	555730	0.36
	2000	0	0	1	1	0	2	555730	0.36
Sweden	1999	1	3	1	0	0	5	518532	0.96
	2000	N/A	N/A	N/A	N/A	N/A	N/A		
UK	1999	12	6	5	4	5	32	3387800	0.94
	2000	15	10	12	15	9	61	3387800	1.80
EU TOTAL*	**1999**	**43**	**21**	**21**	**8**	**5**	**98**	**11573731**	**0.85**
	2000	**39**	**22**	**23**	**20**	**12**	**116**	**10893291**	**1.06**
Australia	1999	10(10)	8(8)	2(2)	0(0)	0(0)	20(20)	1297534	1.54
	2000	6(4)	2(2)	1(0)	1(1)	0(0)	10(7)	1297534	0.77
Czech Republic	1999	17(17)	18(18)	16(16)	13(13)	14(14)	78(78)	463569	16.83
	2000	14(14)	29(27)	10(10)	16(15)	15(13)	84(79)	452761	18.55
Israel	1999	3	1	2	0	0	6	567000	1.06
	2000	6	0	3	0	0	9	567000	1.59

* Denmark reports only meningitis and is therefore excluded from the EU totals
* Numbers in parentheses indicate cases confirmed by isolation in countries where antigen detection is included

Table 6 : Numbers of cases and incidence (per 100,000 population) of invasive Hib meningitis in children under 5 years by country : 1999 & 2000

Country	Year	< 1 yr	1 yr	2 yrs	3 yrs	4 yrs	Total cases <5 years	Population	Rate
Denmark	1999	1	0	0	0	0	1	344685	0.29
	2000	1	0	0	0	0	1	340593	0.29
Ireland	1999		1	0	0	0	1	250394	0.40
	2000	1	1	0	0	0	2	250394	0.80
Finland	1999	0	0	0	0	0	0	324870	0.00
	2000	1	0	0	0	1	2	324870	0.62
Germany	1999	1	1	4	1	0	7	3947634	0.18
	2000	7	3	2	1	0	13	3947634	0.33
Greece	1999	0	0	0	0	0	0	169648	0.00
	2000	N/A	N/A	N/A	N/A	N/A	N/A		0.00
Iceland	1999	0	0	0	0	0	0	20981	0.00
	2000	0	0	0	0	0	0	20981	0.00
Italy (enhanced)	1999	16	6	9	2	0	33	1147352	2.88
	2000	7	2	4	1	2	16	1147352	1.39
Netherlands	1999	3	0	1	1	0	5	976175	0.51
	2000	3	3	2	0	0	8	983491	0.81
Norway	1999	0	1	0	0	0	1	301963	0.33
	2000	0	0	0	0	0	0	302387	0.00
Portugal	1999	1	0	0	0	0	1	555730	0.18
	2000	0	0	1	0	0	1	555730	0.18
Sweden	1999	0	2	0	0	0	2	518532	0.39
	2000	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
United Kingdom	1999	6	3	2	2	2	15	3387800	0.44
	2000	7	4	5	5	2	23	3387800	0.68
TOTAL	1999	28	14	16	6	2	66	11945764	0.55
TOTAL	2000	27	13	14	7	5	66	11261232	0.59
Australia	1999	6	5	1	0	0	12	1297534	0.92
	2000	5	0	0	0	0	5	1297534	0.39
Czech Republic	1999	13	14	6	6	8	47	463569	10.14
	2000	11	23	5	8	4	51	452761	11.26
Israel	1999	2	0	0	0	0	2	567000	0.35
	2000	4	0	0	0	0	4	567000	0.71

3.3.4 Clinical diagnosis

Meningitis remains the dominant clinical diagnosis amongst cases in children reported each year, and no real change in the distribution of diagnoses occurred over the two years. (Table 7) Other than meningitis, epiglottitis was the next most prominent clinical diagnoses (19% and 18% for 1999 and 2000, respectively), followed by septicaemia (13% and 12% in 1999 and 2000, respectively). The proportion of meningitis was highest in all countries except Greece, where septicaemia/bacteraemia represented a higher proportion. (Table 8) Caution has to be taken, however, with the Greek figures as they refer to a very small number of cases.

The proportion of cases with meningitis was much lower amongst adult cases than in children.(Table 9) Epiglottitis was more common in older children (aged 2-14), than in infants, one year olds and adults. Pneumonia and septicaemia/bacteraemia were more common among adult cases, and pneumonia was an extremely rare diagnosis in children.

Table 7 : Cases of invasive Hib disease by clinical diagnosis and year in children under 15 years of age

Diagnosis	**1999**		**2000**	
Meningitis	138	59.2%	145	55.1%
Epiglottitis	43	18.5%	47	17.9%
Cellulitis	3	1.3%	7	2.6%
Osteomyelitis / septic arthritis	1	0.5%	7	2.6%
Pneumonia	9	3.9%	8	3.0%
Septicaemia / bacteraemia	31	13.3%	32	12.2%
Other	5	2.1%	13	4.9%
Not known	3	1.3%	4	1.5%
TOTAL	**233**	**100%**	**263**	**100%**

Table 8 : Cases of invasive Hib disease in children under 15 years of age by clinical diagnosis and country : 1999 & 2000 combined

Country	**Meningitis**		**Epiglottitis**		**Cellulitis**		**Osteomyelitis/ septic arthritis**		**Pneumonia**		**Septicaemia/ bacteraemia**		**Other**		**Not known**	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%%	No.	%
Australia	18	51%	2	6%	2	6%	0	0%	2	6%	9	26%	1	3%	1	3%
Czech Republic	119	58%	63	31%	0	0%	4	2%	4	2%	14	7%	0	0%	2	1%
Ireland	3	43%	1	14%	0	0%	0	0%	0	0%	3	43%	0	0%	0	0%
Finland	3	60%	0	0%	0	0%	0	0%	0	0%	2	40%	0	0%	0	0%
Germany	23	61%	7	18%	0	0%	0	0%	0	0%	6	16%	2	5%	0	0%
Greece	0	0%	0	0%	0	0%	0	0%	0	0%	1	100%	0	0%	0	0%
Israel	6	35%	0	0%	2	12%	0	0%	6	35%	3	18%	0	0%	0	0%
Italy(enhanced)	52	95%	0	0%	0	0%	0	0%	0	0%	1	2%	0	0%	2	4%
Netherlands	14	89%	0	0%	0	0%	0	0%	0	0%	2	13%	0	0%	0	0%
Norway	1	33%	0	0%	0	0%	0	0%	1	33%	0	0%	1	33%	0	0%
Portugal	1	25%	1	25%	0	0%	0	0%	0	0%	0	0%	0	0%	2	50%
Sweden	2	33%	1	17%	0	0%	1	17%	0	0%	2	33%	0	0%	0	0%
UK	43	41%	14	13%	5	5%	3	3%	4	4%	21	20%	14	13%	0	0%
TOTAL	**285**	**57%**	**89**	**18%**	**9**	**2%**	**8**	**2%**	**17**	**3%**	**63**	**13%**	**18**	**4%**	**7**	**1%**

Table 9 : Cases of invasive Hib disease by clinical diagnosis and age group : 1999 & 2000 combined

Diagnosis	< 1 yr	1 yr	2 yrs	3 yrs	4 yrs	5-9 yrs	10-14 yrs	15+ yrs	NK
Meningitis	92 (67%)	65 (66%)	42 (55%)	29 (45%)	19 (41%)	21 (42%)	4 (44%)	14 (12%)	1 (14%)
Epiglottitis	2 (1%)	12 (12%)	18 (23%)	23 (36%)	16 (35%)	18 (36%)	1 (11%)_	12 (10%)	1 (14%)
Cellulitis	5 (4%)	1 (1%)	1 (1%)	1 (2%)	0 (0%)	1 (2%)	1 (11%)	1 (1%)	0 (0%)
Osteo/SA	3 (2%)	2 (2%)	2 (3%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	4 (3%)	0 (0%)
Pneumonia	5 (4%)	3 (3%)	2 (3%)	0 (0%)	3 (7%)	1 (2%)	1 (11%)	26 (22%)	1 (14%)
Septicaemia	24 (18%)	10 (10%)	8 (10%)	7 (11%)	5 (11%)	6 (12%)	2 (22%)	32 (28%)	1 (14%)
Other	2 (1%)	4 (4%)	4 (5%)	3 (5%)	2 (4%)	3 (6%)	0 (0%)	17 (15%)	0 (0%)
Not known	4 (3%)	2 (2%)	0 (0%)	0 (0%)	1(2%)	0 (0%)	0 (0%)	10 (9%)	3 (43%)
All diagnoses	137	99	77	64	46	50	9	116	7

3.3.5 Non capsulated H. influenzae infection

The incidence of non-capsulated invasive H. influenzae disease in children under fifteen was similar to that of type b infection and showed little change between years.(Table 10) This emphasises the importance of accurate identification of strains of *H. influenzae* in children. The range of incidence observed, however, ranged widely between countries, suggesting that ascertainment may be more variable than for type b infections. In view of the technical expertise required to identify non-capsulate infections and the varying use of national reference centres described in the laboratory questionnaire, this is perhaps not surprising.

3.3.6 Other capsulated serotypes of H.influenzae

Compared to both type b and non-capsulate infections, invasive disease due to other capsulated organisms was rare. (Table 11) Type f infections were the most common serotype observed and little change occurred between years of the study.

Table 10 : Incidence of non-capsulated and type b *H. influenzae* in children under 15 years of age–1999 & 2000

Country	Year	Non capsulated	Incidence	Type b	Incidence	Population
Denmark*	1999	1	0.10	1	0.1	967643
	2000	1	0.10	1	0.1	981148
Finland	1999	1	0.11	3	0.32	943001
	2000	1	0.11	2	0.21	943001
Germany	1999	7	0.05	13	0.10	12897014
	2000	28	0.21	25	0.19	12897014
Iceland	1999	2	3.09	0	0	64711
	2000	0	0.0	0	0	64711
Ireland	1999	0	0.0	4	0.47	859424
	2000	0	0.0	3	0.35	859424
Italy (enhanced)	1999	1	0.03	36	1.00	3595194
	2000	0	0.0	19	0.53	3595194
Netherlands	1999	19	0.65	8	0.27	2915911
	2000	7	0.24	8	0.27	2915911
Norway	1999	7	0.79	2	0.23	882408
	2000	6	0.68	1	0.11	882408
Portugal	1999	1	0.06	2	0.11	1744602
	2000	2	0.11	2	0.11	1744602
UK	1999	39	0.39	36	0.36	10033595
	2000	55	0.55	68	0.68	10033595
EU TOTAL*	**1999**	**77**	**0.23**	**104**	**0.31**	**33935860**
	2000	**99**	**0.29**	**128**	**0.38**	**33935860**
Israel	2000	1	0.06	11	0.67	1638400

*Denmark reports only meningitis and is therefore excluded from the EU totals

Table 11 : Other *H. influenzae* serotypes in children under 15 years: all countries combined : 1999 & 2000

Year	Type a	Type c	Type e	Type f	Non-b
1999	1	0	3	10	1
2000	4	2	1	13	3

4. CONCLUSIONS

Prior to introduction of Hib vaccination programmes the epidemiology of invasive Hib disease differed between the EU countries, with incidence rates in children under five varying between 12 and 60 per 100,000. The only country in this collaboration with no vaccination programme has demonstrated incidence rates in the same range (17/100,000). All EU countries now have national immunisation programmes, and therefore the incidence in children under five years, the age group with the highest incidence pre-vaccine, is now very low. Countries are at different stages of vaccine implementation, have different vaccines and schedules and have acheived different levels of coverage. Despite all of these considerations, the incidence of Hib infection in the EU is much lower than in the pre-vaccine era (between 0 and 3.0 per 100,000).

Although the incidence has fallen in countries using vaccine, the clinical presentation of Hib disease has not changed. Meningitis remains the predominant diagnosis, causing over 65% of disease in under two year olds, with epiglottitis being the second most common diagnosis in pre-school children. Pneumonia and bacteraemia are more common presentations in adults. Apparent differences between countries may be explained by the different age distribution of cases and the small numbers of cases.

Amongst children under five in the EU countries, the highest incidence rates in 1999 were in Ireland and Italy. Rates were also high in Australia. These countries were amongst those reporting the lowest coverage in the previous project (funded under DGX11). No updated data on coverage has yet become available (as this was being collected via the EU-VAC project) but an increase in coverage in Australia (personal communication Peter McIntyre) is likely to have explained the reduction observed in that country in 2000.

In 2000, the highest incidence was observed in the UK, who experienced almost a doubling of the number of cases and this increase has continued during 2001 (personal communication, Dr Mary Slack). One of the major differences between the UK and Ireland and the remaining EU countries is the absence of a booster (third or fourth dose) in the second year of life. Although Ireland has a comparatively high rate, no obvious trend has been observed in 2000. Rates between years in each participant country vary due to small numbers but the increase observed in the UK and Germany, two of the largest populations under surveillance, was responsible for an overall increase in incidence in the EU in 2000. The increase in Germany, however, was of a similar magnitude to that observed in the UK, despite the use of a booster vaccination in that country.

Despite the increase in Germany in 2000, the rate remains lower than that described in the UK. Differences in surveillance or in pre-vaccine epidemiology may explain some of this difference. Change in vaccination programmes have occurred over time and may be responsible for changes in incidence observed. In particular, the change from using Hib alone or in combination with DTwP to using combinations with DTaP has occurred in many countries (including Germany but not including the UK). As this combined vaccine is associated with lower post-vaccination antibody levels to Hib, it is important to continue to monitor Hib incidence with this new vaccine. It is unlikely that the increase observed so far can be explained by changes in the vaccine, and if a change in vaccine was implicated, we would expect to see increases in many EU countries. The importance of continued observation over the whole of the EU is therefore essential to ensure that such changes can be detected at the earliest possible stage.

Surveillance systems varied slightly amongst the participating countries. As most countries include all invasive Hib disease in children under fifteen years, comparison of rates in under fives and under fifteens can be made. Differences may be explained by many factors, including different methods of surveillance and completeness of ascertainment. One of the most important factors is the microbiological practice in relation to the diagnosis of Hib disease. This practice can impact on the establishment of disease burden and on comparisons between countries. If laboratories in some countries do not routinely test blood cultures or specimens from other sterile sites for *H. influenzae* in cases with clinical disease compatible with Hib infection then *H. influenzae* and Hib disease will not

be diagnosed. The importance of continued improvement of laboratory techniques and laboratory based surveillance cannot be over-emphasised.

Rates of non-b capsulated *H. influenzae* infection are low and no evidence of capsule-switching has been observed despite many years of vaccination in many of the EU countries. Rates of non-capsulate infection are now similar to those for type b and emphasises the importance of ensuring accurate identification of the organism in a national reference centre. The low rates observed in some countries, probably reflects the low proportion of strains that are referred and highlights the potential for improving ascertainment of such cases. Information on the underlying variability in rates of non-capsulate infection are not known, but the ability to detect such infections may be a useful indicator of the quality of microbiological services in that country. This indicator may help to validate observations of changes in Hib incidence.

5. PROJECT ACHIEVEMENTS

This project has made considerable contributions to :

1. improving the epidemiological information on *Haemophilus influenzae* within the EU ;
2. improving the laboratory capacity of countries within the EU to accurately identify the isolates of *H. influenzae ;*
3. forming a focus for wider collaboration with non European Union countries and candidate European Union countries.

5.1 Improvements in the epidemiological information on *H. influenzae* within the EU

A combination of tools were used to improve the epidemiological information on H. influenzae within the EU. The surveillance system questionnaires from participant countries have allowed greater understanding of the data supplied by each country and have helped explain any limitations in the data supplied. Agreed usage of a minimum dataset and a standardised case definition for *H. influenzae* has enabled valid comparisons to be made of the disease epidemiology between member countries, and hence assist the monitoring of epidemiological changes within Europe. Information collected on the vaccination programme(s) in use by each participant country has also aided interpretation of the epidemiological analyses. The standardisation of laboratory methods used in identification of *H. influenzae* isolates also contributes significantly to the comparability of the epidemiological information between EU countries.

5.2 Improvements in the laboratory capacity within the EU to accurately identify *H. influenzae* isolates

These improvements were achieved through gaining information on systems in use by participant countries, by running a laboratory workshop for new members to the network, and by undertaking an External Quality Assurance Scheme (EQAS) with the participant reference laboratories. Questionnaires completed by network members on the laboratory methods used in the identification of *H. influenzae* gave information that, and, as with the surveillance system questionnaire results, allowed greater understanding of any limitations that could impact on the data individual countries supplied. The EQAS helped identify any existing problems in correctly serotyping *H. influenzae* isolates, and enabled corrections/assistance in laboratory methods to be made, hence improving comparability of data between countries. The laboratory workshop run for new members ensures standardised methods are being used, adding further to correct identification of isolates within the EU.

5.3 Forming a focus for wider collaboration with non European Union countries and candidate European Union countries

Through establishment of this *H. influenzae* disease surveillance network in the European Union, with its standardised case definition, minimum dataset, laboratory workshop skill-sharing and laboratory quality assurance scheme, a focus for wider collaboration with non-EU and candidate EU countries is provided. Involvement of the Israel, Australia, the Czech Republic in this collaboration has increased the population under surveillance. It is hoped that other non-EU countries will join the collaboration later – some are already part of the meningococcal disease network.

6. APPENDICES

6.1 Appendix 1 : *H. influenzae* surveillance network collaborators

AUSTRIA

Reference laboratory

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Email sigrid.heuberger@sime.com

Epidemiology

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FM for Social Security & Generations
Div VIII/D/2
Radetzkystr 2
A-1031 Vienna, AUSTRIA
Tel: -43-71174367 Fax: -43-1-718-71-83
Email: reinhild.strauss@bmsg.gv.at OR reinhild.strauss@uibk.ac.at

BELGIUM

Reference laboratory

Dr Francoise Crokaert
Institut Jules bordet
Laboratoire
rue Heger-Bordet, 1
B-1000 Bruxelles, BELGIUM
Tel: -32-2-541-3700 OR -32-2-541-3706 Fax: -32-2-541-3295
Email: fcrokaert@usa.net

Epidemiology

Dr Frank Van Loock
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DENMARK

Reference laboratory

Dr Helle Bossen Konradsen
Head of Department of Respiratory Infections, Meningitis and STIs
Statens Serum Institut
Artillerivej 54
2300 Copenhagen S, DENMARK
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Email: HBK@ssi.dk

Epidemiology

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Dept of Epidemiology
Statens Serum Institut
Artillerivej 5
2300 Copenhagen S
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Email: STG@ssi.dk & SSM@ssi.dk

FRANCE

Reference laboratory

Pr Henri Dabernat
Centre National de Reference des HI
Laboratoire de Microbiologie
CHU de Purpan
31059 Toulouse
FRANCE
Tel: -33-5-61-77-21-22 Fax: -33-5-61-77-23-33
Email: DABERNAT.H@chu-toulouse.fr

Epidemiology

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Institut de Veille Sanitaire
12 rue du val d'Osne
94415 Saint-Maurice Cedex, FRANCE
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FINLAND

Reference laboratory

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FIN-90101, Oulu, FINLAND
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Tel (Maija): -358-8-537-6235 Fax: -358-8-537-6251
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Epidemiology

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GERMANY

Reference laboratory

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Children's Hospital
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Epidemiology

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GREECE

Reference laboratory

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Aghia Sophia Children's Hospital
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Epidemiology

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ICELAND

Reference laboratory

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Epidemiology

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Division of Infectious Disease Control
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IRELAND

Reference laboratory

Dr Mary Cafferkey
Consultant Microbiologist
Meningococcal Reference Laboratory

Children's Hospital
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Epidemiology
Dr Darina O'Flanagan
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Sir Patrick Dunne's Hospital
Lower Grand Canal Street
Dublin 2
IRELAND
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Reference laboratory
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LUXEMBOURG

Reference laboratory

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NETHERLANDS

Reference laboratory

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Epidemiology

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NORWAY

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Epidemiology

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PORTUGAL

Reference laboratory & Epidemiology

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PORTUGAL
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SPAIN

Reference laboratory & Epidemiology

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SWEDEN

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Epidemiology

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NON-EU COUNTRIES

AUSTRALIA

Reference laboratory

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Prof Lyn Gilbert
Microbial Diagnostic Unit
ICPMR & new Children's Hospital
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Westmead Hospital
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Epidemiology

Dr Peter McIntyre
National Centre for Immunisation & Surveillance
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ISRAEL

Reference & Epidemiology

Professor Ron Dagan
The Paediatric Infectious Disease Unit
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CZECH REPUBLIC

Reference laboratory

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6.2 Appendix 2 : Minimum dataset

Variable name	**Further description**	Field type	Coding
Country		Text	
Year		Number	
IDNO	Identification numbers/letters	Text	
INIT	Initials	Text	
Firstname		Text	
DOB	Date of birth	DD/MM/YY	
DOO	Date of onset	DD/MM/YY	
AgeYr1	Age in years	Number	
Agemth	Age in months if <1 year	Number	
Sex		Number	1=male 2=female 3=not known
Geog	Geographical area/region	Text	
Clin	Clinical diagnosis	Number	1=meningitis 2=epiglottitis 3=cellulitis 4=osteomyelitis/septic arthritis 5=pneumonia 6=septicaemia 7=other (specify in 'OthClin') 9=not known
OthClin	Other clinical diagnosis, if specified	Text	
Method of confirmation		Number	1=culture 2=antigen 3=clinical diagnosis 9=not known
Antigen	*H. influenzae* antigen test positive for type b	Number	
Othisol	Other method of confirmation, if specified	Text	
Site	Site of specimen	Number	1=blood 2=CSF 3=blood & CSF 4=other invasive 5=not relevant 6=other (non invasive) 7=other (not known) 8=other (Ag)
OthSite	Other site, if specified	Text	
Serotype	Serotype if known	Text	B = *H. influenzae* type b A = *H. influenzae* type a C = *H. influenzae* type c E = *H. influenzae* type e F = *H. influenzae* type f NC = *H. influenzae* non-capsulated/not typeable NT = *H. influenzae* un-typed NK = not known
Vacc	Vaccination status	Number	1= vaccinated 2=not vaccinated

			3=not applicable 4=not known
Doses	No. of doses of vaccine given pre-onset	Text	99=not known
VF	Vaccine failure	Text	TVF = True Vaccine Failure AVF = Apparent Vaccine Failure PVF = Possible Vaccine Failure
Dose1	Vaccine type	Text	
Date1	Date given	DD/MM/YY	
Dose2	Vaccine type	Text	
Date2	Date given	DD/MM/YY	
Dose3	Vaccine type	Text	
Date3	Date given	DD/MM/YY	
Boost	Booster vaccine type	Text	
Bdate	Date booster given	DD/MM/YY	
Outcome		Number	1=alive 2=died 3=not known

6.3 Appendix 3 : H. influenzae Surveillance systems questionnaire

Hib Vaccination in Europe - Invasive *Haemophilus influenzae* infections

Surveillance systems questionnaire

Country: .....
Name of respondent: .....
Position: .....
Centre: .....
Address: .....
.....
.....

The purpose of this questionnaire is to describe the current surveillance systems for *Haemophilus influenzae* in your country and to provide comparative information for each participating country.

Notes for completion of questionnaire
Please complete Part A **once** for overall *H. influenzae* surveillance.
Please complete Part B for **each** surveillance system.
Please attach any additional information/reports.

Part A

1 Surveillance methods

1.1 Methods

What methods of surveillance of *Haemophilus influenzae* are used in your country?
(please list the methods used and complete Part B of the questionnaire once for each system)

1.2 Data collation

If more than one system: How is the data collated at a national or regional from each system?

- ☐ Individual case reconciliation*
- ☐ Comparison of aggregate data only
- ☐ No collation of systems
- ☐ Not relevant

* "reconciliation" - cases in one system merged with cases in another system and duplicates removed.

For each method of surveillance please complete one questionnaire Part B.

Part B

1 Surveillance system

1.1 Objectives

What are the objective(s) of this *Haemophilus influenzae* surveillance system method? (please specify if the system aims for sentinel or universal case ascertainment)

1.2 Case definitions

What is the case definition or case category of the health event under surveillance?

H. influenzae type b ☐ *H.influenzae* ☐ Other ☐
Please specify "Other" .....
.....

Meningitis ☐ All invasive ☐ Other ☐
Please specify "Other" .....
.....

1.3 Population

What is the population under surveillance?

Whole country ☐ Region ☐ Please specify which region(s)
.....

Total population ☐
Under 15 years of age ☐
Under 10 years of age ☐
Under 5 years of age ☐
Other (specify) .....

1.4 Type of surveillance system

What type of surveillance system is this?

Type of system
Active ☐
Passive ☐

Characteristics of system
Stimulated ☐ Not stimulated ☐
Statutory reporting ☐ Voluntary reporting ☐

Zero-reporting ☐ / No zero reporting ☐

1.5 Start of surveillance system

Which year did this surveillance system start? .....
Years for which data is available .....

2 Data collection

2.1 Information collected

What information/data is collected?
(please specify the variables routinely collected)

Age ☐
Sex ☐
Date of onset ☐
Geographic location ☐
Clinical condition ☐
Organism ☐
Method of confirmation ☐
Vaccination status ☐
Other ☐ Please specify "Other" .....
.....
.....

2.2 Reporting sources

Who provides the data? (please specify who reports the data used)
Clinicians ☐
Paediatricians ☐
Microbiologists ☐
Epidemiologists ☐
Scientific staff ☐
Administrative staff ☐
Other, please specify .....

Where is the data received from?
Hospitals ☐
Clinics ☐
Reference laboratory ☐
Local laboratories ☐
Other, please specify .....
.....

2.3 Time period

How frequently is the data reported locally?
Weekly ☐ Monthly ☐ Quarterly ☐
Six-monthly ☐ Annually ☐ Other .....….

How frequently is the data aggregated nationally?
Weekly ☐ Monthly ☐ Quarterly ☐
Six-monthly ☐ Annually ☐ Other .....

2.4 Duplicate reports

Are duplicates routinely detected and eliminated?

3 Data analysis

3.1 Analysis

Who analyses the data at a national level?

Clinicians ☐
Paediatricians ☐
Microbiologists ☐
Epidemiologists ☐
Scientific staff ☐
Adminstrative staff ☐
Other, please specify .....
.....

4 Data dissemination

4.1 Regular reports

4.1a Frequency

How often are reports of the surveillance system produced?
(please state this for all regular reports)

Weekly ☐

Monthly ☐
Quarterly ☐
Six-monthly ☐
Annually ☐
Other .....

4.1b Method of reporting

How are the reports disseminated?
(please state if this is by bulletin, website, newsletter, etc)

4.1c Audience

Who are reports disseminated to?

4.2 Recent publications

Are there any recent or relevant publications demonstrating application(s) of the surveillance system?
And Are there any recent or relevant publications about evaluation(s) of the system and/or changes in the system?
(please list any recent or relevant publications)

6.4 Appendix 4 : Laboratory diagnostic methods questionnaire

Hib Vaccination in Europe - Invasive *Haemophilus influenzae* infections

Laboratory Diagnostic Methods Questionnaire

Country :.....

Name of respondent .....

Position .....

Centre .....

Address .....
.....
.....
.....

The first section aims to describe the facilities which are available in the hospitals which refer strains to you.

The purpose of the second section is to describe the methods used to identify H.influenzae by laboratories collaborating in this study.

Please return both sections of completed questionnaire to:-

Dr. Mary P.E. Slack
Haemophilus Reference Laboratory
Public Health Laboratory,
Level 6/7, John Radcliffe Hospital,
Headington,
Oxford, OX3 9DU
U.K.

(Tel: +44-1865-220879/220884 Fax: +44-1865-220890)

SURVEY OF LABORATORY FACILITIES FOR THE IDENTIFICATION OF HAEMOPHILUS INFLUENZAE IN.....

I)What proportion of hospitals in your country/area have the facilities to do the primary identification of H.influenzae strains?

100%	☐
80-100%	☐
50-80%	☐
20-50%	☐
<20%	☐

II) For those hospitals which can identify H.influenzae, what type of cases/specimens would they look for/try to grow the organism from?

All CSFs from suspected bacterial meningitis	☐
All CSFs from suspected bacterial meningitis in children	☐
All blood cultures	☐
All blood cultures in children	☐
Blood cultures from cases of epiglottitis	☐
Blood cultures from cases of epiglottitis in children	☐
Other conditions, please describe	☐
(e.g. osteomyelitis, septic arthritis, pneumonia)	_____

III) What proportion of hospitals would be able to perform serotyping on isolates of :

H.influenzae type b

100%	☐
80-100%	☐
50-80%	☐
20-50%	☐
<20%	☐

Other H.influenzae

100%	☐
80-100%	☐
50-80%	☐
20-50%	☐
<20%	☐

IV) What proportion of hospitals refer isolates to the reference lab (i.e. your lab)?

100%	☐
80-100%	☐
50-80%	☐
20-50%	☐
<20%	☐

V) For those hospitals which do refer isolates to your lab, what type of cases are they referred for?

All invasive H.flu	☐
All invasive H.flu in children	☐
H.flu meningitis	☐
H.flu meningitis in children	☐
H.flu epiglottitis in children	☐
Other, please describe	☐

REFERENCE LABORATORY METHODS

1.1 Receipt of strains

		yes	no
1.11	Are the strains subbed immediately on receipt?	☐	☐
1.12	Are the strains tested on receipt, or batched?	☐	☐
1.13	Are the strains stored and tested in batches?	☐	☐

2.1 Media

2.11 What media is used to transport strains to the laboratory?
.....……

2.12 What media is used to subculture the strains?
.....

2.13 What media is used to test growth factor requirement?
.....

2.14 What media is used for susceptibility testing?
.....

2.15 What media is used for long term storage of strains?
.....

2.16 Please state atmosphere of incubation.
.....

2.17 Please state duration of incubation.
.....

2.2 Identification Methods

Are the following tests performed? (Please tick the appropriate box)

	yes	no
Catalase	☐	☐
Oxidase	☐	☐
Dependence on growth factors		
i) by disc method	☐	☐
ii) by plate incorporation method	☐	☐
Porphyrin	☐	☐

Satellitism on blood agar yes ☐ no ☐
(please state origin of blood used i.e. horse, sheep) .....
.....

Haemolysis yes ☐ no ☐
(please state origin of blood used).....
.....

Nitrate yes ☐ no ☐
If Yes, please state method
.....
.....

O.N.P.G. yes ☐ no ☐

Commercially available identification kit yes ☐ no ☐
(Please give details).....
.....
.....

Other, please specify yes ☐ no ☐
.....
.....

2.3 Are the strains biotyped using the following tests?

Indole	yes	☐	no	☐
Urease	yes	☐	no	☐
Ornithine decarboxylase	yes	☐	no	☐

2.4 Are the strains serotyped?

If so, which of the following methods are used:

Slide agglutination with polyvalent antisera yes ☐ no ☐
If yes, give details of antisera used
.....
.....

Slide agglutination with type specific antisera yes ☐ no ☐
If yes, give details of antisera used
.....

Counter current immunoelectrophoresis yes ☐ no ☐
PCR yes ☐ no ☐
If yes, give details of primers used
.....
.....

Other yes ☐ no ☐
If yes, give details
.....

2.5 Are the strains further subtyped? yes ☐ no ☐

If yes, which typing method is used?

OMP ☐
Ribotyping ☐
LPS ☐
PFGE ☐
Other, please specify ☐

2.6 Susceptibility testing.

2.6.1 Please list antimicrobial chemotherapeutic agents tested, and concentrations (e.g. disc content, breakpoint values, etc.)
.....
.....
.....
.....
.....
.....

2.6.2 With method of susceptibility testing is used?

	yes	no
Disc diffusion - please state method e.g.		
Control organism on the same agar plate		
Control organism on a separate agar plate		
Break points		
Other, please specify		

2.6.3 If MICs are required, which method is used?

	yes	no
Broth dilution		
Agar incorporation		
E-test (AB BIODISK)		
Commercially prepared MIC microtitre trays		
If so, please give details of kit used		
.....		
Other		
Please specify		
.....		
.....		

2.7 Do you test for beta-lactamase production? yes ☐ no ☐

If yes, please state method used

.....

.....

.....

2.8 Do you test for chloramphenicol acetyltransferase (CAT) production? yes ☐ no ☐

If yes, please state method used

.....

.....

.....

2.9 Long term storage

How do you store strains long term?

	yes	no
Agar slopes		
Frozen at -80oC		
Other		

Please specify .....

.....

Please give any other information regarding your laboratory methods not covered above. (Please attach additional sheets if necessary, or include your laboratory standard operating procedures)