Epidem. Inf. (1987) 99, 591–601
Printed in Great Britain

The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*

BY K. A. V. CARTWRIGHT,

Public Health Laboratory, Great Western Road, Gloucester GL1 3NN

J. M. STUART,

Department of Community Medicine, Rikenel, Gloucester GL1 1LY

D. M. JONES

Public Health Laboratory, Withington Hospital, Manchester M20 8LR

AND N. D. NOAH

PHLS Communicable Disease Surveillance Centre, Colindale, London NW9 5EQ

(*Accepted 18 August 1987*)

SUMMARY

A total of 6234 nasopharyngeal swabs was collected during a survey of the population of Stonehouse, Gloucestershire in November 1986 as part of an investigation into an outbreak of meningococcal disease. The overall meningococcal carriage rate was 10·9%. The carriage rate rose with age from 2·1% in the 0- to 4-year-olds to a peak of 24·5% in the 15- to 19-year-olds, and thereafter declined steadily with age. Male carriers outnumbered female carriers of meningococci by 3:2. Group B (or non-groupable) type 15 sulphonamide-resistant strains which had caused the outbreak were isolated from 1·4% of subjects. The age distribution of carriers of these strains was similar to that of other meningococci apart from an additional peak in the 5–9-year age group and a more rapid decline in carriage with increasing age. Variations in the carriage rates of the outbreak strain were seen in children attending different schools and in the residents of different areas of the town. The low carriage rate of these strains in a community during a prolonged outbreak supports the hypothesis that these organisms are less transmissible but more virulent than other strains of pathogenic meningococci.

Carriage of *Neisseria lactamica*, which is thought to be important in the development of meningococcal immunity, was most frequent in children under the age of 5 years and was six times commoner in this age group than carriage of *Neisseria meningitidis*. In older children and adults female carriers of *N. lactamica* increasingly outnumbered males in contrast to the male preponderance observed with meningococcal carriage.

INTRODUCTION

In November 1986 a large-scale community survey was undertaken in the town of Stonehouse, Gloucestershire during an outbreak of meningococcal disease due to group B type 15 p1.16 sulphonamide-resistant (B15.16R) strains in Gloucester Health District (Stuart *et al.* 1987). During this outbreak 15 out of 94 cases had occurred in Stonehouse (population 6635).

Smaller-scale surveys during this and other outbreaks in London and Somerset revealed low nasopharyngeal carriage rates of B15.16R strains among contacts of cases (Cartwright, Stuart & Noah, 1986; Cann *et al.* 1987; C. Bowie, personal communication). The combination of the sustained high attack rate seen in outbreaks due to B15.16R strains (Poolman *et al.* 1986) and the low carriage rate in contacts suggests that this meningococcal strain may be of relatively high virulence but with a low rate of transmission.

Two of the primary objectives of the Stonehouse investigation were to measure more accurately the prevalence of nasopharyngeal carriage of B15.16R strains and to compare the geographical distribution of these strains both with that of other meningococci and with that of cases in a community experiencing active but localized disease due to B15.16R strains. Observations were also made on the nasopharyngeal carriage rate of *Neisseria lactamica*.

MATERIALS AND METHODS

Nasopharyngeal swabs were collected as previously described (Stuart *et al.* 1987). A New York City formulation (available from authors) was used for the isolation medium with final concentrations of 5% lysed horse blood and 10 mg per litre of trimethoprim, vancomycin and colistin. Plates were freshly prepared at Bristol Public Health Laboratory (PHL) and each batch was tested for the ability to support the growth of the outbreak strain. Plates were inoculated directly and taken by van in alternate batches to Gloucester and Bristol PHLs where they were incubated in 5% CO_2 for a minimum of 48 h. All colonies resembling meningococci were identified by conventional tests (oxidase, Gram reaction, microscopic morphology and ONPG reduction) and sent in batches on Dorset egg slopes for grouping and typing to the Meningococcal Reference Laboratory (MRL), Manchester. Cultures were maintained at the primary laboratories until the growth of all strains at the MRL was confirmed. Strains of *Neisseria lactamica* identified by the same set of tests were submitted in batches on Dorset egg slopes to Hereford PHL for freeze drying.

On receipt at the MRL meningococci were subcultured to blood agar and on the following day suspensions were made in saline and the group of the organisms determined by co-agglutination. From the same suspensions sulphadiazine and rifampicin minimum inhibitory concentrations were determined. Groups B, C and non-groupable meningococci were subcultured to Kelloggs medium for subsequent serotyping by co-agglutination using monoclonal antibodies. Non-groupable strains were confirmed as meningococci by reactions in cystine trypticase agar carbohydrates. Short-term storage of all meningococci was on Dorset agar at 30 °C and all sulphonamide-resistant strains were also stored at −70 °C in glycerol broth.

To check the sensitivity of detecting meningococci by postnasal swabbing 202 consecutive attenders consented to a second swab immediately after the first. The two swabs were taken by different nurses and were processed in the usual way. To establish the reproducibility of grouping, typing and sensitivity testing 40 strains of meningococci were submitted twice to the MRL with altered identification numbers. These validations were undertaken without the knowledge of the relevant laboratory staff.

The isolation of many sulphonamide-resistant non-groupable (NG) strains expressing serotype 15:P1.16 antigens prompted a study to ascertain whether these strains were phenotypically identical to B15.16R strains apart from the expression of group polysaccharide. Individuals from whom B15.16R meningococci had been isolated were swabbed again 1 month after the main survey. If on the second examination growth from the confluence on the primary plates was group B, five separate colonies were subcultured, grouped and serotyped.

RESULTS

During the 2-week survey period 6234 nasopharyngeal swabs were collected from 5006 Stonehouse residents and 1228 non-resident school-children and staff at Stonehouse schools. The overall meningococcal carriage rate in Stonehouse residents was 10·9% (Table 1). Only seven (2·1%) children under 5 years of age were found to be carrying meningococci; the age-specific carriage rate for all meningococci rose to a maximum of 24·5% in the age group 15–19 years before declining steadily with increasing age.

Of 24 individuals carrying group B15.16R meningococci again at 1 month follow-up, 6 showed phenotypic variation amongst the organisms selected. Five showed variation in the grouping result, with the simultaneous presence of both B15.16R and NG15.16R strains suggesting that such strains are identical except for the expression of capsular polysaccharide. One carrier had B non-typable and NG 15 phenotypes suggesting either that two organisms were present or that variation in the expression of serotype antigens may also occur in isolates from the nasopharynx. Polyacrylamide gel electrophoresis of the pairs of B15.16R and NG15.16R phenotypes gave indistinguishable polypeptide patterns. A selection of these strains was also shown to belong to the same clone by starch gel electrophoresis (R.A. Wall, personal communication). We therefore designated as an 'outbreak' strain any group B or non-groupable meningococcus which was sulphonamide-resistant and which expressed the type 15 antigen with or without the p1.16 subtype antigen.

Outbreak strains were carried by 69 (1·4%) residents tested (Table 1); there was a great diversity of other groups and types amongst the 476 other strains of meningococci isolated (Table 2). If the age distributions of carriers of outbreak and other strains of meningococci are compared two main differences are apparent (Figure 1). Children in the 5–9-year age group carried 14·5% of the outbreak strains compared with 4·8% of the other meningococci ($P < 0·05$) whereas those aged 55 and over carried 1·4% of the outbreak strains compared with 16·2% of the other meningococcal strains ($P < 0·001$). The sex distribution showed a predominance of male carriers in all meningococcal groups including outbreak strains (Table 3). The overall ratio of male to female carriers was 1·5 to 1.

Table 1. *Meningococcal carriage rates and cases in Stonehouse residents by age*

Age in years	No. tested	All meningococcal carriers		Outbreak strain carriers		No. of cases
		No.	(%)	No.	(%)	
0–4	328	7	(2·1)	1	(0·3)	2
5–9	421	33	(7·8)	10	(2·4)	7
10–14	367	36	(9·8)	6	(1·6)	4
15–19	388	95	(24·5)	11	(2·8)	0
20–24	386	71	(18·4)	10	(2·6)	0
25–34	812	103	(12·7)	14	(1·7)	1
35–44	678	78	(11·5)	11	(1·6)	0
45–54	442	44	(10·0)	5	(1·1)	1
55–64	510	44	(8·6)	1	(0·2)	0
65+	674	34	(5·0)	0	(0·0)	0
Total	5006	545	(10·9)	69	(1·4)	15

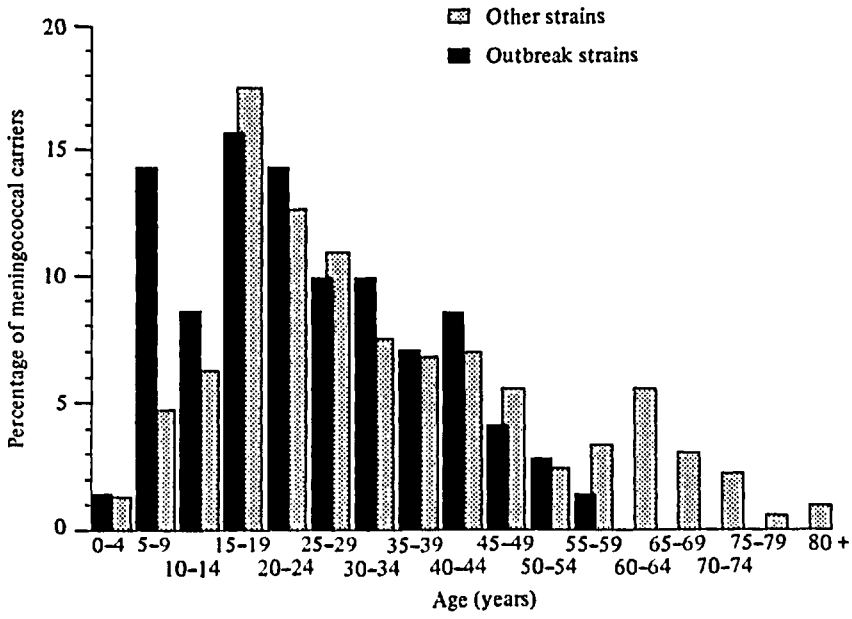

Fig. 1. Comparative age distribution of outbreak and other strains of meningococci.

Residents of Park Estate and Verney Fields where 14 of the 15 cases lived had significantly higher meningococcal carriage rates overall than residents of the remainder of the town [329/2489 (13·2%) compared with 216/2517 (8·6%), $P < 0·001$] (Table 4). Although Park Estate and Verney Fields also had high carriage rates of the outbreak strain the highest carriage rate was found in Bridgend where there had been no cases. The remaining two areas of the town had significantly lower carriage rates of the outbreak strain than Park Estate and Verney Fields ($P < 0·001$). The distribution of non-groupable outbreak strains did not differ significantly from the distribution of group B outbreak strains when analysed by age, sex or area of residence, nor was there any apparent clustering of outbreak strains within areas.

Table 2. *Meningococci in Stonehouse residents by serogroup and serotype*

Group	Type	Sulphonamide sensitivity	Number
	Outbreak strains		
B	15	R	5
B	15:P1.16	R	32
NG	15	R	2
NG	15:P1.16	R	30
	Other strains		
A	N	R	1
	N	S	2
B	15	S	15
	15:P1.16	S	1
	2a	S	3
	2a:P1.2	S	1
	2b	S	2
	2b:P1.3	S	2
	2c	S	1
	P1.16	S	8
	P1.2	S	14
	P1.3	S	6
	nt	R	3
	nt	S	98
NG	15	S	27
	15:P1.16	S	4
	2b	S	1
	P1.16	S	15
	P1.2	S	38
	P1.3	R	2
	P1.3	S	17
	nt	R	8
	nt	S	94
C	15	S	1
	2b:P1.2	S	1
	P1.16	S	1
	P1.3	S	2
	nt	R	2
	nt	S	12
29E, W135, X, Y, Z	N	S	88
	N	R	6
Total			545

Table 3. *Sex distribution of meningococcal carriage in Stonehouse residents*

Strain	Male	Female	Ratio M:F
Outbreak	41	28	1·5:1
Other B	97	57	1·7:1
Other NG	124	84	1·5:1
29E, A, C, W135, X, Y, Z	62	52	1·2:1
Total	324	221	1·5:1

https://doi.org/10.1017/S0950268800066449 Published online by Cambridge University Press

Table 4. *Meningococcal carriage rate and cases by area of residence in Stonehouse*

Area	No. tested	All meningococcal carriers		Outbreak strain carriers		No. of cases
		No.	(%)	No.	(%)	
Park Estate	1273	165	(13·0)	25	(2·0)	11
Verney Fields	1216	164	(13·5)	18	(1·5)	3
Little Australia	750	62	(8·3)	5	(0·7)	0
Rosedale	1226	99	(8·1)	5	(0·4)	1
Bridgend	541	55	(10·2)	16	(3·0)	0
Total	5006	545	(10·9)	69	(1·4)	15

Table 5. *Meningococcal carriage rates and cases in Stonehouse schools*

School	No. of pupils tested	All meningococcal carriers		Outbreak strain carriers		No. of cases
		No.	(%)	No.	(%)	
State infant/junior, (Day)	441	35	(7·9)	12	(2·7)	8
Private junior (a) (Boarding)	49	0	(0·0)	0	(0·0)	—
Private junior (b) (Day)	180	25	(13·9)	0	(0·0)	—
State secondary (Day)	736	110	(14·9)	13	(1·8)	1
Private senior (Boarding)	331	53	(16·0)	1	(0·3)	—
Special (Day)	51	3	(5·9)	0	(0·0)	1
Total	1788	226	(12·6)	26	(1·5)	10

Four of the 69 outbreak strains were found in household members of previous cases, all of whom had received rifampicin prophylaxis at the time of illness of the index case. Meningococci were not isolated during the survey from any of the three cases recorded subsequently, although one of these cases occurred in a household where outbreak strain carriers had been found and treated.

Carriage rates in the schools showed a difference in pattern between outbreak and other strains (Table 5). Of the 1177 pupils tested at the state primary and secondary schools 25 (2·1%) were found to be carrying the outbreak strain compared with only one carrier of this strain in the 560 pupils sampled from the three private schools ($P < 0·01$). Four out of 241 (1·7%) children resident in Stonehouse who went to school outside Stonehouse were found to be outbreak strain carriers. Carriage rates of other meningococci showed no consistent differences between state and private schools. No serogroup or serotype was consistently found in the classes of any of the schools or in the residents of dormitories at the private schools. Among 247 school staff tested only one (a member of staff at one of the private schools) was found to be carrying an outbreak strain.

https://doi.org/10.1017/S0950268800066449 Published online by Cambridge University Press

Table 6. *Carriage rate of* Neisseria lactamica *by age and sex in Stonehouse residents*

Age	No. tested	Male	Female	Total (%)
0–4	328	29	17	46 (14·0)
5–9	421	12	8	20 (4·8)
10–14	367	6	5	11 (3·0)
15–19	388	4	7	11 (2·8)
20–24	386	2	8	10 (2·6)
25–34	812	12	15	27 (3·3)
35–44	678	3	4	7 (1·0)
45–54	442	1	5	6 (1·4)
55–64	510	0	5	5 (1·0)
65+	674	0	4	4 (0·6)
Total	5006	69	78	147 (2·9)

Table 7. *Isolation of meningococci from postnasal swabbing*

Agreement in paired swabs	
Both culture negative	180
Both culture positive (same strain)	18
Total	198
Disagreement in paired swabs	
One culture negative, one culture positive	3
Both culture positive (different strain)	1
Total	4
Concordance	98%

The overall carriage rate of *N. lactamica* was 2·9% with a peak in children aged under 5 years (Table 6). In children aged less than 15 years male carriers outnumbered females by 1·6:1. From age 15 onwards females increasingly outnumbered males.

No gonococci were isolated from any of the 6234 swabs.

Of the 202 pairs of swabs taken by different nurses 198 yielded the same culture results, a concordance of 98% (Table 7). If two negative swabs are taken to constitute a true negative (i.e. no meningococcal colonization of the nasopharynx) and one positive swab a true positive (i.e. meningococci are present in the nasopharynx) then sensitivity of one nasopharyngeal swab in the detection of meningococci was 93%.

Of the 40 cultures submitted twice to the MRL there was a concordance of 95% for group and sulphonamide sensitivity results and 81% for serotype result (Table 8). All but one of the differences in group or type were due to variation in the expression of a particular antigen either polysaccharide (group) or protein (type). There were no instances of any change from one antigen to another, e.g. group B to group C or type 2 to type 15.

Table 8. *Validation of laboratory testing for meningococcal group, serotype and sulphonamide sensitivity*

Group			Concordance
Agreement	both NG	17	
	both same group	21	
Total		38	
Disagreement	A to NG	1	
	NG to Y	1	
Total		2	95%
Serotype			
Agreement	both non-typable	14	
	both same type	11	
Total		25	
Disagreement	15 to non-typable	4	
	non-typable to 15	1	
	15 to P1. 16	1	
Total		6	
Not tested		9	81%
Sulphonamide sensitivity			
Agreement	both sensitive	37	
	both resistant	1	
Total		38	
Disagreement	S to R	1	
	S to ?	1	
Total		2	95%

DISCUSSION

The size of this survey permitted an accurate analysis of the distribution of meningococcal groups and types by age, sex and area of residence in a civilian community. The principal findings relating to all meningococci were a low carriage rate in children under 5 years, a peak in young adults and a declining carriage rate with advancing age. The low carriage rate in young children contrasts with the usual peak incidence of meningococcal disease in this age group (Greenwood, 1984). The gradual decline in carriage rate with increasing age may reflect progressive acquisition of immunity in the nasopharynx to meningococci, decreasing physiological suitability of the nasopharyngeal environment or reduced likelihood of acquisition due to decreasing close social contact.

Only 69 Stonehouse residents carried outbreak strains out of a sample population of 5006, suggesting that unless there is a very short period of carriage these strains are not easily transmitted from person to person. The relatively high proportion of children in the 5–9 age group carrying outbreak strains coincided with the peak of meningococcal disease seen in Stonehouse, 7 of the 15 cases having occurred in this age group. On the other hand in two other groups with high carriage rates of outbreak strains (in those aged 15–19 and 20–24) no cases occurred. By contrast with the carriage of other meningococcal strains, only a single outbreak strain was isolated from 1184 subjects sampled over the age of 55. If declining meningococcal carriage rates in the elderly are due to lower frequency

of close social contact, this finding is consistent with the suggested low transmissibility of outbreak strains.

The sex distribution of carriers of outbreak strains followed the same pattern as that seen for carriers of other meningococci with males outnumbering females by 3:2. A male excess has also been reported in cases of meningococcal disease in Gloucestershire (Cartwright, Stuart & Noah, 1986) and in Britain generally (Abbott *et al.* 1985; Fallon, Brown & Lore, 1984).

A striking feature of meningococcal disease in Stonehouse since 1983 has been the localization of cases in residents of two housing estates (Park Estate and Verney Fields). These were also the areas with the highest carriage rates for all meningococci and with the highest proportion of council-owned housing (Stuart *et al.* 1987). Although there was no consistent relationship between density of population and meningococcal carriage rates, previous evidence suggests that overcrowding must be severe before affecting carriage rates (Kaiser *et al.* 1974). High carriage rates of outbreak strains were also found in Park Estate and Verney Fields but the highest carriage rate of these strains was found in residents of Bridgend. No residents of Bridgend have had meningococcal meningitis in the current outbreak. The proportion of the population under the age of 16 years in Bridgend is marginally lower than the other two areas and the lower overall meningococcal carriage rates suggest that acquisition rates may be lower.

Most cases of disease had occurred in children attending the state infant or junior school and it was therefore not unexpected to find the highest carriage rate of outbreak strains amongst children at these schools, 97% of whom were Stonehouse residents. Pupils at the state secondary school, of whom 41% lived in Stonehouse, had similar carriage rates of outbreak strains to Stonehouse resident children who attended schools outside Stonehouse. Only 8% of pupils at the private schools lived in Stonehouse and social interaction between pupils and residents of the town is limited. This may explain the low carriage rate of outbreak strains amongst these pupils when compared with children attending the state schools.

In the age, school and area data it was apparent that in this population there were three patterns of infection with outbreak strains. In one, the highest rate of carriage of these strains was associated with a high case rate, in the second group similar rates of carriage were not accompanied by a high case rate and in the third both carriage and case rates were very low. This therefore reinforces the view that high carriage rates are important but are not the only factor determining disease in a population.

No comparably large survey of postnasal carriage of *N. lactamica* had previously been undertaken. Earlier studies have shown that peak carriage rates of *N. lactamica* occur in young children (Gold *et al.* 1978) and our study confirms this finding. This survey shows in addition a reversal in the sex ratio of carriers of *N. lactamica* with increasing age. The adult female carrier excess may be related to contact with young children in whom the organism is most frequently found, first as mothers and then later as grandmothers. The importance of these organisms is that colonization with them may induce antibodies which cross-react with and protect against pathogenic meningococci (Gold *et al.* 1978). Meningococcal disease

in young children may occur in those who encounter pathogenic strains of meningococci prior to being colonized with *N. lactamica*.

The survey lasted for 2 weeks and in order to collect postnasal swabs from over 6000 persons during this period more than 20 different nurses and doctors were employed in the collection of these specimens. High concordance in the duplicate swabbing tests shows that postnasal swabbing is a sensitive procedure in the detection of meningococci. It also suggests that carriers of meningococci usually have substantial numbers of organisms on the posterior pharyngeal wall; this is supported by the abundant growth of meningococci seen on most of the primary isolation plates. Scanty positives were unusual.

One pair of swabs yielded what were apparently two different organisms – a non-groupable, non-typable sulphonamide sensitive strain and a B 15: P1.16 sulphonamide-resistant organism. However the failure to detect two different organisms in any other paired swabs suggests that different strains do not usually co-exist in the nasopharynx unless one strain is greatly predominant. The only way to detect multiple co-existing strains would be to group and type a substantial number of individual colonies from positive postnasal swabs.

Reversion from expression of group or type antigen to a non-groupable or non-typable state did not appear to be dependent on the age of the culture, i.e. strains when first isolated were no more likely than a week-old subculture to express group or type antigen in detectable amount. The importance of detection of these antigens lies not only in their epidemiological significance but also in their contribution to the virulence of the strain. Meningococcal strains isolated from cerebrospinal fluid or from blood agglutinate more easily than strains collected from the patient's nasopharynx at the same time, suggesting that organisms well-endowed with capsular polysaccharide are more pathogenic.

The hypothesis that B15.16R strains are less transmissible than other meningococci was proposed to explain the prolonged nature of the outbreaks in Norway and in Gloucestershire. An outbreak which continues for many years implies continuing availability of susceptible individuals and this suggests that the causative organism is only moving slowly through the community. We have established that there is a low carriage rate in a defined community at a time of high disease activity. If the B15.16R strain is only passed from person to person with difficulty and is yet capable of sustaining a prolonged outbreak it is unusually virulent. Elucidation of its virulence factors may give insights into mechanisms of meningococcal pathogenicity.

These epidemiological characteristics of a prolonged outbreak accompanied by a low carriage rate of the outbreak strain also suggest that this strain has a relatively long duration of carriage. In the control of meningococcal outbreaks mass swabbing of contacts is not usually considered worthwhile (N.D. Noah, personal communication). If outbreaks are caused by an organism with a low carriage rate, long duration of carriage and high virulence it may be justifiable to swab more widely and to give antibiotic treatment to all those found to be carriers of the outbreak strain. This strategy was recently followed in a London school (Cann *et al.* 1987). We are undertaking further investigations to examine both the duration of carriage and the effectiveness of antibiotic treatment in eliminating carriage of the B15.16R strain.

We are very grateful to Mrs E. Sutcliffe for undertaking the organization of the serogrouping and the phenotype studies, to Mrs J. Eldridge for the serotyping of the meningococcal isolates and to all the staff of the PHLS who helped so much in managing the substantial workload arising from this study. We also thank Professor J. R. T. Colley for helpful comments on the manuscript. This study was supported by a DHSS grant.

REFERENCES

Abbott, J. D., Jones, D. M., Painter, M. J. & Young, S. E. J. (1985). The epidemiology of meningococcal infections in England and Wales 1912–1983. *Journal of Infection* **11**, 241–257.

Cann, K. J., Rogers, T. R., Jones, D. M., Noah, N. D. & Burns, C. (1987). *Neisseria meningitidis* in a primary school. *Archives of Disease in Childhood*. In press.

Cartwright, K. A. V., Stuart, J. M. & Noah, N. D. (1986). An outbreak of meningococcal disease in Gloucestershire. *Lancet* ii, 558–561.

Fallon, R. J., Brown, W. M. & Lore, W. (1984). Meningococcal infections in Scotland 1972–82. *Journal of Hygiene* **93**, 167–180.

Gold, R., Goldschneider, I., Lepow, M. L., Draper, T. F. & Randolph, M. (1978). Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. *The Journal of Infectious Diseases* **137**, 112–121.

Greenwood, B. M. (1984). Meningococcal Infection. In *Oxford Textbook of Medicine* (ed. Weatherall, Ledingham & Warrell), 5, 165–174.

Kaiser, A. B., Hennekens, C. H., Saslaw, M. S., Hayes, P. S. & Bennett, J. V. (1974). Sero-epidemiology and chemoprophylaxis of disease due to sulphonamide resistant *Neisseria meningitidis* in a civilian population. *The Journal of Infectious Diseases* **130**, 217–224.

Poolman, J. T., Lind, I., Jonsdottir, K., Froholm, L. O., Jones, D. M. & Zanen, H. C. (1986). Meningococcal serotypes and serogroup B disease in north-west Europe. *Lancet* ii, 555–557.

Stuart, J. M., Cartwright, K. A. V., Jones, D. M., Noah, N. D., Wall, R. J., Blackwell, C. C., Jephcott, A. E. & Ferguson, I. R. (1987). An outbreak of meningococcal disease in Stonehouse: planning and execution of a large scale survey. *Epidemiology and Infection* **99**, 579–589.