Epidemiol. Infect. (1998), **121**, 495–505. Printed in the United Kingdom

Acquisition and carriage of meningococci in marine commando recruits

T. RIORDAN[1], K. CARTWRIGHT[2]*, N. ANDREWS[3], J. STUART[4], A. BURRIS[2], A. FOX[5], R. BORROW[5], T. DOUGLAS-RILEY[6], J. GABB[6] AND A. MILLER[7]

[1] *Public Health Laboratory, Church Lane, Heavitree, Exeter EX2 5AD*
[2] *Public Health Laboratory, Gloucestershire Royal Hospital, Great Western Road, Gloucester GL1 3NN*
[3] *PHLS Statistics Unit, 61 Colindale Avenue, London NW9 5EQ*
[4] *PHLS CDSC South & West, Public Health Laboratory, Gloucestershire Royal Hospital, Great Western Road, Gloucester GL1 3NN*
[5] *PHLS Meningococcal Reference Unit, Public Health Laboratory, Withington Hospital, Manchester M20 8LR*
[6] *Commando Training Centre, Royal Marines, Lympstone, Devon EX8 5AR*
[7] *Royal Naval Hospital, Gosport, Hants PO12 2AA; now: Kidderminster General Hospital, Bewdley Road, Kidderminster, Worcestershire DY11 6RJ*

(*Accepted 18 July 1998*)

SUMMARY

Meningococcal acquisition is a prerequisite for invasive disease. Three hundred and eleven male marine commando recruits were studied throughout 29 weeks of basic training to identify factors influencing meningococcal carriage and acquisition including troop number, season, smoking, respiratory infection, antibiotic usage and nasopharyngeal bacterial interference flora.

A high carriage rate on entry to training (118/311, 37·9%) and subsequent sustained high rates of meningococcal acquisition were found. Of the potential factors examined, only active and passive smoking were found to be associated significantly with meningococcal carriage on entry. The association between active smoking and meningococcal carriage was dose-dependent, with odds ratios (OR) of 2·2 (95% CIs 1·0–4·8) and 7·2 (95% CIs 2·3–22·9) for light and heavy smokers respectively. Passive smoking predisposed independently to carriage (OR 1·8, 95% CIs 1·1–3·0). Active and passive smoking combined to give an attributable risk for meningococcal carriage of 33%. In contrast, despite a high and sustained rate of meningococcal acquisition in the study population, none of the risk factors investigated, including active smoking, was associated significantly with meningococcal acquisition. No cases of meningococcal disease occurred during the 16-month study period. Therefore smoking may increase the duration of meningococcal carriage rather than the rate of acquisition, consistent with the increased risk of meningococcal disease from passive as opposed to active smoking. Public health measures that reduce the prevalence of smoking should reduce the risk of meningococcal disease.

INTRODUCTION

Meningococcal disease remains a significant cause of morbidity and mortality in children and young adults in the UK [1] and elsewhere [2]. Since recent acquisition of a pathogenic strain of meningococcus is a prerequisite for invasive disease [3], factors favouring acquisition are important in the pathogenesis of the infection. Point prevalence studies of carriage are frequently used as a proxy for meningococcal acquisition, but if the duration of meningococcal

* Author for correspondence.

carriage varies [4–8], the relationship between acquisition and carriage is not necessarily predictable.

There are many published studies of meningococcal carriage [9]. Carriage rates peak in young adults, and are low in the very young and the elderly [10]. Male sex [10, 11], cigarette smoking [11–13] and severe overcrowding [13, 14] are all associated with increased rates of carriage. Caugant and colleagues, studying a normal (i.e. non-epidemic) population, found in addition that those working outside the home, especially in transportation or industry, were more likely to harbour meningococci [11].

The available data indicate that season [7, 15, 16], and by inference, respiratory tract infections, have little or no influence on carriage rates. However, Olcén and colleagues, studying household contacts of meningococcal disease, recorded a higher prevalence of symptoms of upper respiratory infection among meningococcal carriers than non-carriers [17]. Interference by competing bacterial strains among the normal flora of the upper respiratory tract may also affect meningococcal carriage [18]. Treatment of meningococcal carriers with antibiotics such as rifampicin may result in freedom from meningococcal carriage for months or possibly even years [19].

Evidence from prospective studies [3] and from laboratory-acquired infections (K. Cartwright, unpublished data; D. M. Jones and J.-Y. Riou, personal communications) suggest that when invasive meningococcal disease occurs, the interval between acquisition of a pathogenic meningococcus and systemic invasion is normally brief – a matter of a few days. However, studies of factors affecting acquisition, which require repeated swabbing of cohorts of individuals, have been reported less frequently than carriage studies [5, 17, 15, 20].

In military recruit camps large numbers of young adult males, a group with a high meningococcal carriage rate, are brought into close proximity for periods of several months, favouring enhanced transmission of meningococci. An increased risk of cases and clusters of meningococcal disease in military recruit camps has been recognized since the last century [21]. Over 70 years ago, Glover showed that acquisition and carriage rates in such camps could be very high, the latter exceeding 50% from time to time, and that periods of very high carriage rates were associated with development of cases of invasive infection [22]. Subsequently, an association between high rates of meningococcal carriage and disease in defined communities has been questioned [23, 24].

Preliminary studies in a United Kingdom Royal Marine recruit training centre had shown high levels of meningococcal carriage and therefore, by inference, of acquisition. This suggested that the centre would serve as a suitable location in which to try to elucidate further the relative contributions of a number of possible factors influencing meningococcal acquisition and carriage. The influence of meningococcal acquisition and carriage on serum and salivary anti-meningococcal outer membrane protein (OMP) antibodies was also investigated, but the findings of these studies will be reported separately.

SUBJECTS, MATERIALS AND METHODS

Recruit training

At the time of the study, new troops of 30–40 Royal Marine recruits began basic training at the Commando Training Centre, Lympstone, Devon, at 4-weekly intervals throughout the year. Training normally lasted for 29 weeks. For the first fortnight, members of a troop all slept in one large dormitory. Subsequently recruits were quartered in smaller rooms sleeping 6–8 persons. The training programme involved considerable physical exercise and included arduous physical endurance tests each of which had to be passed before a recruit could continue with the next stage of training. Approximately 25% of those starting the programme failed to complete it, and a further 25% lost time through injury or illness such that they were 'back-trooped' and completed training with a later troop than that in which they started. At the time of the study, recruits were not offered meningococcal vaccine on entry to training.

Specimen collection and initial processing

Permission to carry out the study was granted by the Royal Navy Ethical and Research Committee and by the Commanding Officer of the Commando Training Centre. Specimen collection at the Training Centre was carried out between January 1994 and May 1995. During the first week of training all recruits attended the sick-bay for medical examination and immunization. The background to the study and the implications of taking part were explained to recruits by one of the camp's medical officers. Entry to the study was voluntary and recruits were included only if they gave written consent.

Pairs of plain cotton swabs were used to sample the posterior pharynx of all participating recruits on entry and then again at 4-weekly intervals throughout training. Swabbing was by the trans-oral route [25]. One of each pair of swabs was plated directly onto Oxoid (Oxoid Ltd, Basingstoke, UK) New York City neisserial selective agar (containing colistin sulphate 7·5 mg/l, trimethoprim 3·0 mg/l, vancomycin 2·0 mg/l and amphotericin 1·0 mg/l). Plates were transported to Exeter Public Health Laboratory (PHL) within 1 h and incubated at 37 °C in 5% CO_2 for 48 h.

Presumptive meningococci were identified by colonial morphology, Gram stain and oxidase reaction. All isolates were sub-cultured to chocolate agar slopes for transmission to the Meningococcal Reference Unit (MRU) at Manchester PHL. At the MRU, isolates were sub-cultured onto blood agar and incubated in 5% CO_2 for 24 h. On the following day, suspensions were made and isolates speciated, serogrouped by co-agglutination [26], and serotyped and sero-subtyped [27].

Interference with meningococcal growth by nasopharyngeal aerobic commensal flora

The second of each swab pair was inoculated directly onto tryptone soya agar (TSA) and Columbia blood agar base (COL). TSA was used as a test medium on the basis of work done by Filice and colleagues [18], and COL was selected following preliminary investigations at Gloucester PHL.

Agar plates were transported directly to Gloucester PHL where they were incubated overnight in 5% CO_2 at 37 °C. Semi-quantitative estimation of the total aerobic nasopharyngeal flora (normal flora) was made on a scale of $\pm$ to $+++$. Interference with the growth of a serogroup A meningococcus by organisms present in the nasopharyngeal flora was then detected using a modification of a previously described agar overlay technique [28]. *N. meningitidis* (Haj strain – A4:P1·9, supplied by the MRU), was grown overnight at 37 °C on COL supplemented with 5% v/v defibrinated horse blood (BA). This strain was selected following preliminary comparative studies of representative strains of different meningococcal serogroups. Filice and colleagues also used a serogroup A strain. A standardized suspension of meningococci in tryptone water was added to 500 ml molten (50 °C) Mueller–Hinton agar (MH) to a final concentration of approximately 10^6 c.f.u./ml). Inoculated overlay medium (7·5 ml) was then pipetted onto the surface of each plate growing nasopharyngeal organisms. Plates were again incubated overnight.

The next day, inhibition of meningococcal growth (i.e. interference flora) was recorded in a semi-quantitative fashion, on a scale of $\pm$ to $+++$. Individual colonies causing inhibition were picked with a straight wire through the overlay and sub-cultured onto BA. The ability of these isolates to inhibit the growth of the Haj meningococcus was confirmed by retesting. The picked organism was spot-inoculated onto TSA and COL, incubated overnight and then overlaid with Haj meningococcus as described above. An isolate was considered to be inhibitory if meningococcal growth was again clearly visibly inhibited over the site of inoculation of the test organisms.

By converting the $\pm$ to $+++$ scale for interference flora on COL and TSA to a numerical scale of 0·5–3·0, an average value was calculated for abundance of interference flora in each swab.

Sporadic contamination of TSA and COL plates by *Bacillus* spp. occurred. When these plates were overlaid with MH agar a single *Bacillus* sp. colony would invariably spread over the whole surface of the agar obscuring any interference effect. Approximately 3% of swabs were affected. Results of these swabs were excluded from the analysis.

Immunological studies

Samples of clotted blood and saliva were obtained from recruits at the start, midpoint and end of training (weeks 1, 15 and 29). Samples were transported to Exeter PHL where serum was separated. Serum and saliva samples were refrigerated prior to transmission by post to the MRU. Serum samples were tested for meningococcal OMP IgG and IgM antibody using an ELISA test as previously reported [29].

Personal health and lifestyle factors

A questionnaire was used for collection of data on smoking and on wife/girlfriend/partner status. This allowed categorization firstly by active smoking history into non-smoker (no tobacco smoked), light smoker (up to 10 cigarettes per day) and heavy smoker (more than 10 cigarettes per day) and secondly

https://doi.org/10.1017/S0950268898001496 Published online by Cambridge University Press

by passive smoking history through exposure to smoking either by household members or partners.

Data on recent respiratory infections and on recent exposure to antibiotics was based on self-reporting at swabbing sessions. Neither data on sick-bay attendance nor details of specific antibiotics administered were recorded.

Meningococcal carriage and acquisition

The following definitions were used:

Carrier: a recruit from whose nasopharynx a meningococcus was isolated on at least one occasion.

Non-carrier: recruits from whom at least three nasopharyngeal swabs were collected, all of which were negative for meningococci on culture.

Strain acquisition: culture of a meningococcus from a recruit preceded immediately by two or more consecutive negative nasopharyngeal swabs, and with no prior meningococcus isolated.

Statistical methods

Associations between potential risk factors and carriage on entry were examined using logistic regression in the statistical package GLIM4 [30]. Factors examined included troop number, season, active smoking, passive smoking, history of recent respiratory infection, history of recent antibiotic usage and presence and amount of pharyngeal bacterial interference flora. Factors were tested individually in single variable models before examination in multivariable models.

Cox's proportional hazards model was used in the statistical package STATA [31] to examine risk factors for meningococcal acquisition. Factors tested were as listed above for carriage, but with the addition of back-trooping, normal flora level, troops' initial prevalence of smoking and meningococcal carriage and pre-existing serum meningococcal OMP antibody. Only those recruits with negative meningococcal cultures on both swabs 1 and 2 were enrolled in the acquisition study model. Time to acquisition, or to a final negative result were modelled, and the various risk factors were tested for their effect on acquisition. Factors were tested individually in single variable models and those with a P value of 0·25 in a multivariable model.

A further analysis was carried out using the proportion of swabs positive per recruit as the outcome measure. Only recruits with three or more swabs were included in this analysis. Risk factors tested were troop number, back-trooping status, active smoking status on entry, girlfriend/wife status, passive smoking status, occurrence of two or more respiratory infections during training, self-reported recent antibiotic use and mean levels of normal and interference flora. This analysis permitted the exploitation of data which might otherwise have been overlooked, since analysis by acquisition employed data only up to the point of acquisition, and analysis by carriage on entry tested only those factors relevant to the results of meningococcal culture of the first swab. Normal regression was used after adjustment of the proportions with an arc-sine transformation to enable a valid analysis.

Troop carriage rates for different meningococcal serogroups were compared using χ^2 tests or Fisher's exact test.

RESULTS

Age structure of recruits population and compliance with specimen collection

A total of 311 recruits in 8 troops participated in the study. Troops ranged in size from 29 to 46 (mean 39). Recruits were all young adult males (mean age 20 years). Among recruits, 79 (25%) were back-trooped on at least one occasion. The median number of swabs per recruit was 7. A total of 114 recruits had all 8 swabs collected throughout training as planned. A further 24 recruits had 9 or 10 swabs as a result of back-trooping and re-entry to the study. Eighteen percent (56/311) of recruits had fewer than 3 swabs.

Meningococcal carriage on entry (Fig. 1)

On entry, 188 of 311 (37·9%) recruits were meningococcal carriers. Rates ranged from 27·5% in troop 655, the first troop to commence training, to 53·8% in troop 660. Differences in carriage rates between troops on entry to training (Fig. 1) were not statistically significant ($P = 0·52$). There was no evidence of a seasonal effect on meningococcal carriage rate.

Risk factors for carriage on entry (Tables 1, 2)

Questionnaires were completed by 294/311 recruits (94·5%) on entry. Of 49 current smokers (16·6%), 31 were light, and 18 were heavy smokers.

https://doi.org/10.1017/S0950268898001496 Published online by Cambridge University Press

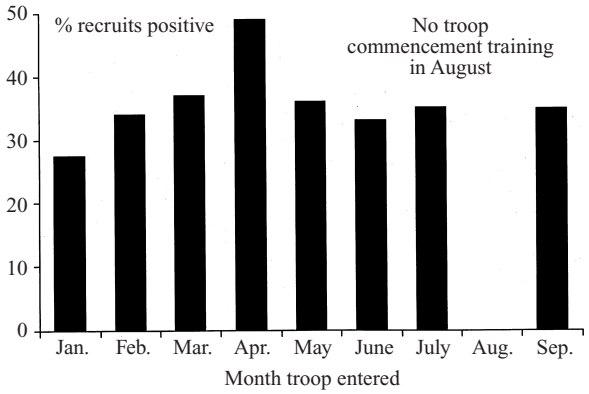

Fig. 1. Carriage rate of entry by month of commencement of training.

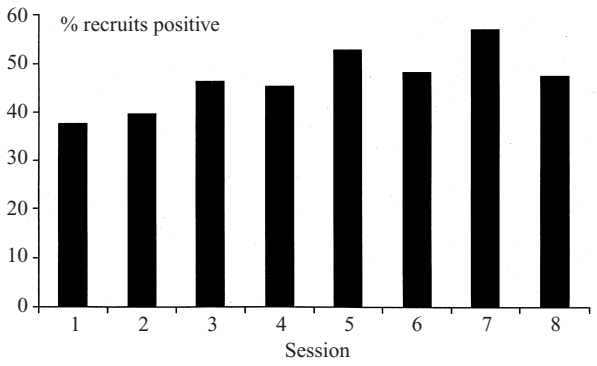

Fig. 2. Carriage rates by session throughout training.

Results from the single variable analysis are shown in Table 1. Troop number, recent respiratory infection, recent exposure to antibiotics and presence of interference flora in the nasopharyngeal swab were not significantly associated with carriage. Recruits recently exposed to antibiotics were less likely to be carriers, with an OR of 0·69, but this trend did not reach significance; only small numbers of recruits had been exposed recently to antibiotics.

When active and passive smoking were examined together in a multivariable model, both were associated with an increased likelihood of meningococcal carriage (Table 2). The association between active smoking and meningococcal carriage was dose-related. There was an independent effect of passive smoking, demonstrating that whether or not a recruit was a smoker, his odds of carriage increased if he was also exposed passively to cigarette smoke. Hence heavy smokers also exposed to smoking had an OR for meningococcal carriage of 13·0 ($7{\cdot}2 \times 1{\cdot}8$). The independent attributable risks were estimated as 14% for active, and 19% for passive smoking. This could explain 33% of meningococcal carriage.

Analysis of risk factors by proportion of swabs positive for meningococci

Three factors were associated significantly with the proportion of positive swabs in a multivariable normal regression analysis. As might be expected, recent exposure to antibiotics was associated with a lower proportion of positive swabs ($P = 0{\cdot}01$), whereas the occurrence of more than two respiratory infections was associated with a higher proportion of positive swabs ($P = 0{\cdot}02$). Surprisingly, a higher mean interference flora level was also associated with a higher proportion of positive swabs ($P = 0{\cdot}005$).

Fitted values using these three risk factors gave estimates for the proportion positive ranging from 28% (two or fewer infections, low levels of interference flora and recent exposure to antibiotics) to 84% (more than two infections, high levels of interference flora, no recent antibiotic exposure).

Sequential carriage data (Fig. 2)

Data on carriage rates for troops by session (i.e. sequentially through training) are shown in Figure 2. Mean carriage rates ranged from 37·9% at session one to a peak of 56·8% at session seven. The χ^2 test for trend showed a significant upward trend ($P < 0{\cdot}001$).

Serogroup data (Fig. 3)

After exclusion of multiple isolates from the same individual, meningococcal strains which could not be serogrouped predominated amongst all isolates, followed by strains of serogroup B. Serogroup C strains were isolated only rarely throughout the study. The prevalence of strains of serogroups X and Y rose in late sessions at the expense of serogroup B and non-groupable strains, reflecting their spread through the members of individual troops, e.g. Troop 655 (Fig. 4). This trend was significant ($P < 0{\cdot}01$).

Meningococcal acquisition

Fifty-six recruits were excluded from the analysis of strain acquisition because fewer than three swabs were obtained. A further 115 were excluded because they grew a meningococcus from swab 1 or 2; of these 115, 19 (17%) went on to acquire a new strain and 96 (83%) acquired no new strains after swab 2.

https://doi.org/10.1017/S0950268898001496 Published online by Cambridge University Press

Table 1. *Single variable analysis of risk factors for carriage*

Risk factor	Factor level	No. of recruits	% Carriers on first swab	Odds ratio	95% CIs	*P* value
Troop	655	40	27·5	1·00	Baseline	0·52
	656	29	37·9	1·61	0·58–4·47	
	658	32	37·5	1·58	0·58–4·28	
	660	39	53·8	3·07	1·20–7·84	
	662	40	37·5	1·58	0·61–4·06	
	663	40	37·5	1·58	0·61–4·06	
	664	45	37·8	1·60	0·64–4·01	
	666	46	34·8	1·41	0·56–3·53	
Recent respiratory infection	No	216	37·9	1·00	Baseline	0·99
	Yes	95	37·9	1·00	0·61–1·64	
Recent antibiotics	No	291	38·5	1·00	Baseline	0·48
	Yes	20	30·0	0·69	0·26–1·83	
Interference flora level	0	92	34·8	1·00	Baseline	0·49
	1	69	36·2	1·07	0·56–2·04	
	2	74	45·9	1·59	0·85–2·98	
	3	69	37·7	1·13	0·59–2·17	
Smoking status	Non	246	30·9	1·00	Baseline	0·0003
	Light	31	48·4	2·10	0·99–4·46	
	Heavy	17	76·5	7·27	2·30–23·0	
Passive smoking exposure	No	165	29·7	1·00	Baseline	0·021
	Yes	129	42·6	1·76	1·09–2·85	

Table 2. *Multivariable analysis of selected risk factors for carriage*

factor	Factor level	Adjusted odds ratio	95% CIs	*P* value
Smoking status	Non	1·00	Baseline	0·0003
	Light	2·24	1·04–4·82	
	Heavy	7·18	2·25–22·94	
Passive smoking exposure	No	1·00	Baseline	0·021
	Yes	1·80	1·09–2·97	

Of the remaining 140, 94 acquired a meningococcus and 46 were persistently negative. Figure 5 shows the rate of acquisition per swabbing session for recruits not previously carriers. Acquisitions occurred steadily throughout training among the diminishing numbers of non-carriers and without a significant peak. There were no cases of meningococcal disease identified in participants over the duration of the study.

Risk factors for meningococcal acquisition (Table 3)

Of the 140/311 recruits available for analysis of acquisition, the only factors showing statistical sig-

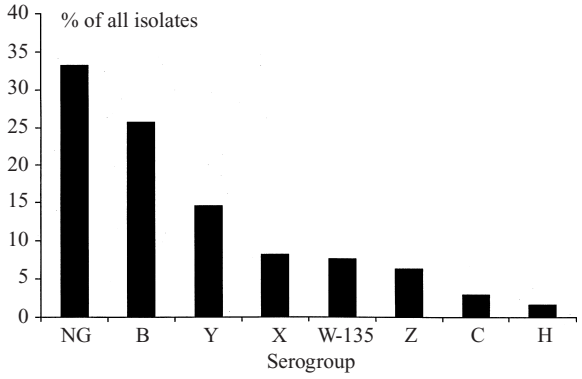

Fig. 3. Meningococcal isolates by serogroup (excludes repeat isolates in the same individual).

https://doi.org/10.1017/S0950268898001496 Published online by Cambridge University Press

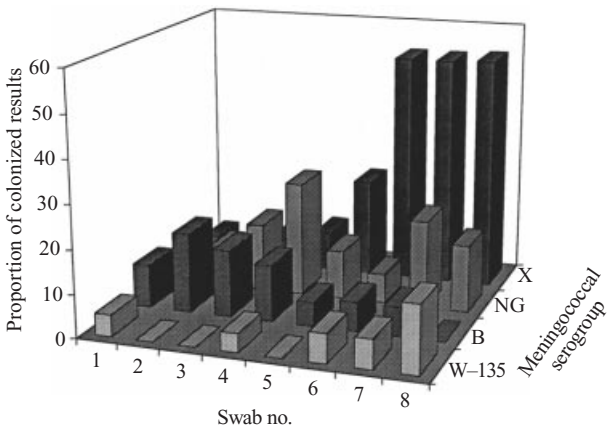

Fig. 4. Carriage rates of meningococcal serogroups in Troop 655 by session.

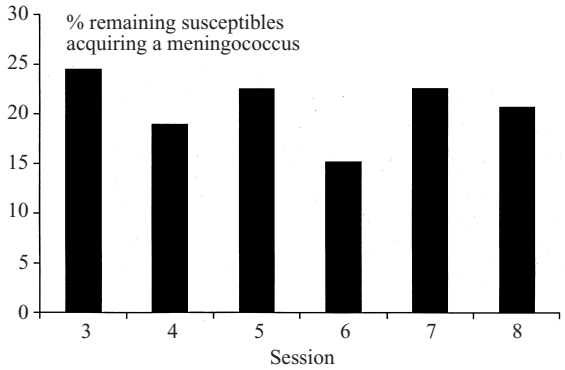

Fig. 5. Acquisition rates by swabbing session (by definition, acquisition could not be detected in the first two sessions).

nificance in single variable analysis were troop number (in particular Troop 655), troops' initial proportion of smokers, and season (Table 3). For many risk factor analyses there were low numbers at some of the factor levels (e.g. numbers of heavy smokers) and hence a low power to detect significant effects. Smoking by recruits in the camp was only permitted in one designated area, and not in accommodation blocks or any other communal areas. The effect of recent antibiotic treatment was as expected (hazard ratio 0·53) but the ratio did not reach statistical significance.

In the multivariable analysis, only membership of Troop 655 had a possible independent effect (hazard ratio compared to all other troops combined of 1·93, 95% CIs 0·98–3·81; $P = 0·06$). The effects of season ($P = 0·29$) and troops' initial proportion of smokers ($P = 0·63$) were no longer close to statistical significance. Troop 655, the first troop in the study, had a low proportion of smokers on entry and had a large number of acquirers on swab 3 (swabs collected in March).

We were unable to demonstrate an effect of season on either carriage or acquisition. Further, there was no effect of pre-existing ELISA meningococcal OMP antibody (IgG or IgM) on risk of meningococcal acquisition.

DISCUSSION

Subjects and study design

The choice of cohorts of recruits in training for this study was based on the fact that recruits are known to have high meningococcal acquisition and carriage rates, thereby facilitating the study of possible risk factors in a non-epidemic setting. The prolonged, structured training programme facilitated sequential sampling at regular intervals. A potential disadvantage of studying this population was that the subjects were not representative of the general population in respect of factors such as gender, sleeping accommodation, the level of physical fitness and of continued exposure to intensive physical exertion. Cigarette smoking rates on entry to training (16·6%) were low; the prevalence of smoking in 16- to 19-year-old males in Great Britain in 1994 was 28% [32]. We did not attempt to gather data on factors such as social class or domestic overcrowding.

Meningococcal carriage on entry

The carriage rate on entry was high at 38%. Meningococcal carriage rates in the UK are known to peak in this age group, and to be higher in males than females [10]. High carriage rates may have reflected efficient swabbing and culture techniques. Detection of meningococci in the nasopharynx on the basis of a single nasopharyngeal swab is known to be insensitive [8] and the carriage rates determined on entry, despite being high, were certain to be underestimates.

Smoking and meningococcal carriage on entry

The data on smoking and carriage on entry confirm and amplify the findings of other studies [11–13, 33]. To date, smoking is the single most powerful identified factor influencing meningococcal carriage. In this study we were able to confirm the independent predictive effects of active and passive smoking in increasing the likelihood of meningococcal carriage. Smoking may have accounted for a third of meningococcal carriage.

https://doi.org/10.1017/S0950268898001496 Published online by Cambridge University Press

Table 3. *Single variable analysis of risk factors for acquisition*

Risk factor	Factor level	Hazard ratio	95% CIs	*P* value
Troop	655	2·36	1·17–4·75	0·067
	656	0·85	0·38–1·90	
	658	1·12	0·48–2·58	
	660	0·69	0·31–1·56	
	662	1·12	0·55–2·38	
	663	0·55	0·20–1·40	
	664	1·24	0·64–2·43	
	666	1·00	Baseline	
Month (season)	Winter (Dec.–Feb.)	1·00	Baseline	0·043
	Spring (Mar.–May)	2·69	1·21–5·97	
	Summer (June–Aug.)	2·28	1·00–5·21	
	Autumn (Sep.–Nov.)	1·72	0·79–3·81	
Back trooper status	No	1·00	Baseline	0·26
	Yes	1·28	0·83–1·96	
Active smoking status	Non-smoker	1·00	Baseline	0·62
	Light smoker	0·90	0·43–1·85	
	Heavy smoker	2·09	0·51–8·62	
Passive smoker on entry status	No	1·00	Baseline	0·22
	Yes	0·77	0·50–1·18	
Recent respiratory infection	No	1·00	Baseline	0·61
	Yes	1·11	0·72–1·71	
Recent antibiotics	No	1·00	Baseline	0·22
	Yes	0·53	0·19–1·48	
Troops' initial prevalence of carriage	Low (< 35%)	1·00	Baseline	0·54
	High (> 35%)	0·87	0·56–1·35	
Percentage of smokers in troop of entry	Low (< 15%)	1·00	Baseline	0·04
	High (> 15%)	0·66	0·44–0·99	
Interference flora level	0	1·00	Baseline	0·54
	1	0·74	0·36–1·50	
	2	0·90	0·46–1·76	
	3	1·10	0·58–2·08	
IgM status on entry	Negative	1·00	Baseline	0·50
	Positive	1·26	0·64–2·53	
IgG status on entry	Negative	1·00	Baseline	0·70
	Positive	0·92	0·60–1·40	

Antibiotics and meningococcal carriage on entry

Exposure to recent antibiotics had no significant effect on meningococcal carriage rates on entry. This may have been due to the fact that the number of recruits who had received antibiotics just prior to entry was small. Also, some exposure might have been to antibiotics without any activity against meningococci. When exposure to antibiotic therapy was compared with the proportion of swabs positive for each recruit, a much larger volume of data could be analysed, and antibiotics were then found to be protective. This association seems logical and has been noted in other studies [34].

Season, respiratory infections and meningococcal carriage on entry

Significant differences between carriage rates on entry to training were not detected. Since troops commenced training at roughly monthly intervals, this finding provided additional evidence for the (somewhat surprising) failure of season to affect meningococcal carriage rates [7, 15, 16]. This suggests that viral infection is unlikely to be an important predisposing factor for meningococcal carriage.

However, recruits who experienced more than two respiratory infections during the study were more likely to have a higher proportion of positive swabs.

https://doi.org/10.1017/S0950268898001496 Published online by Cambridge University Press

This observation, and the finding of a higher carriage rate in household contacts of cases with symptoms of upper respiratory infection [17] are consistent with Pether's hypothesis that meningococci can cause sore throat [35].

Interference flora and meningococcal carriage on entry

The study of interference flora was reliant on methodology which lacked substantial validation with regard to meningococci. Recruits showed a fairly even distribution from no detectable interference flora to level 3, the maximum level. However, not only did we fail to detect any protective effect from the presence of interference flora, but there was a trend, albeit not significant, towards higher carriage rates in recruits with higher interference levels. This possible association was supported by the fact that a high interference flora level was significantly associated with a higher proportion of positive swabs for individual recruits. These observations conflict with the limited published data available [18].

Spread of individual serogroups

Recruits in this study did not receive meningococcal vaccination on entry to basic training. The distribution of meningococcal serogroups amongst recruits on entry was consistent with data from other carriage studies in non-epidemic situations. It was striking that throughout training, serogroup C strains were uncommon with no detectable transmission within troops. In contrast, strains of serogroups X and Y appeared to disseminate rapidly within troops, suggesting substantial variation between meningococcal serogroups in their capacity for dissemination, or variable host resistance to acquisition dependent on the meningococcal serogroup concerned.

Persistent freedom from acquisition or carriage

One of the most striking findings of the study was the fact that despite very heavy exposure to meningococci throughout training, 22/138 (15·9%) recruits who had eight or more swabs remained persistently free of meningococci. However, the monthly acquisition rate amongst residual susceptible recruits remained steady throughout basic training, averaging 21%, with no diminution of acquisitions towards the end of the training period, suggesting that most if not all residual susceptible recruits would have acquired a meningococcus had training continued.

Possible explanations include a failure to detect meningococci sensitively in nasopharyngeal swabs (though carriage rates were very high in this study), a high level of host mucosal resistance to meningococcal colonization, or an undetected inhibitory effect of components of the nasopharyngeal flora (e.g. anaerobic bacteria, which were not sampled in this study). The failure of serogroup A meningococci to spread in UK populations [36] following documented introductions from the Middle East [37] suggests that some part of mucosal resistance to colonization may be serogroup-specific. Such resistance to colonization may be a key component of protection from invasive meningococcal infection.

Meningococcal acquisition

There was a significant increase in carriage rates as the study progressed. Given that there was a substantial incidence of acquisition, this was not unexpected. The high acquisition rate in Troop 655 was probably an artefact, resulting from this being the first troop to enter the study, and with the lowest initial prevalence. Staff may not have gained sufficient swabbing experience at this early stage and swabbing techniques may have been suboptimal for swabs 1 and 2 for this troop.

The decision to require two consecutive negative swabs prior to a positive in order to define strain acquisition was stringent (and based on previous unpublished work); it ensured that our analysis was of true acquisition rather than a consequence of insensitive swabbing. The expectation of a high rate of acquisition in the study population was confirmed. Of 139 initially negative recruits with sufficient swabs taken 92 (66%) acquired a meningococcus during training.

The even distribution of acquisitions throughout the duration of training is at odds with the timing of the known high risk of invasive meningococcal disease in recruits, which occurs principally in the first 3–4 weeks of basic training [38, 39]. The strains circulating within our study population were mainly of low invasive potential (non-groupable strains and strains of serogroups X, Y, W-135 and 29E). In particular, strains of serogroup C were notable for their scarcity; however, there were numerous strains of serogroup B isolated, at least some of which were likely to have had invasive potential.

Our attempts to identify risk factors associated with acquisition of meningococci were unsuccessful. A trend towards protection from acquisition through exposure to antibiotics did not attain significance; only a small number of recruits received antibiotics. As with carriage rates, high levels of interference flora were weakly associated with acquisition. The lack of protection from acquisition by pre-existing IgG or IgM meningococcal OMP serum antibodies was unexpected.

Active smoking was not associated with an increased risk of meningococcal acquisition, despite a strong association with carriage. This surprising finding may be true. However, in this study, the majority of smokers were colonized on entry and there were very few smokers left as potential acquirers. Thus we may have failed to detect a true association. Passive smoking during training was not assessed.

If smokers have a higher prevalence of carriage but are no more likely to acquire meningococci than non-smokers, we infer that the association between smoking and carriage may be caused by prolongation of duration of carriage. Further research is needed, or re-analysis of previous studies, to confirm this point. An increased risk of disease in smokers would not necessarily be expected, since their immunity could be boosted by repeated and prolonged exposure to nasopharyngeal meningococci. In contrast, those in close contact with smokers might be expected to experience an increased risk of acquisition and disease.

This hypothesis is supported by case control studies demonstrating a higher risk of invasive disease in those who live with smokers, and no, or minimally increased risk amongst active smokers themselves [40, 41]. Since asymptomatic carriers are the source of over 95% of meningococcal infections [42], and since smokers are more likely to be carriers, measures which reduce the prevalence of smoking may be expected to reduce the incidence of meningococcal disease.

ACKNOWLEDGEMENTS

We thank the marine commando recruits and their non-commissioned officers who participated in the study, the staff of the Sick Bay, Commando Training Centre, Lympstone, Devon, for their willing and expert collaboration in specimen collection, and the staff of Exeter, Gloucester and Manchester PHLs for taking on additional work during the course of the study.

The study was funded by the UK Department of Health.

REFERENCES

1. Ramsay M, Kaczmarski E, Rush M, Mallard R, Farrington P, White J. Changing patterns of case ascertainment and trends in meningococcal disease in England and Wales. CDR Rev 1997; **4**: R49–54.
2. Hubert B, Caugant D. Recent changes in meningococcal disease in Europe. Eurosurveill 1997; **2**: 69–71.
3. Edwards EA, Devine LF, Sengbusch CH, Ward HW. Immunological investigations of meningococcal disease. III. Brevity of group C acquisition prior to disease occurrence. Scand J Infect Dis 1977; **9**: 105–10.
4. Rake G. Studies of meningococcus infection. VI. The carrier problem. J Exp Med 1934; **59**: 553–76.
5. Greenfield S, Sheehe PR, Feldman HA. Meningococcal carriage in a population of 'normal' families. J Infect Dis 1971; **123**: 67–73.
6. Altmann G, Egoz N, Bogokovsky B. Observations on asymptomatic infections with *Neisseria meningitidis*. Am J Epidemiol 1973; **98**: 446–52.
7. Gold R, Goldschneider I, Lepow ML, Draper TF, Randolph M. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. J Infect Dis 1978; **137**: 112–21.
8. Pether JVS, Lightfoot NF, Scott RJD, Morgan J, Steele-Perkins AP, Sheard SC. Carriage of *Neisseria meningitidis*: investigations in a military establishment. Epidemiol Infect 1988; **101**: 21–42.
9. Cartwright K. Meningococcal carriage and disease. In: Cartwright K, ed. Meningococcal disease. Chichester: Wiley & Sons, 1995: 115–46.
10. Cartwright KAV, Stuart JM, Jones DM, Noah ND. The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. Epidemiol Infect 1987; **99**: 591–601.
11. Caugant DA, Høiby EA, Magnus P, et al. Asymptomatic carriage of *Neisseria meningitidis* in a randomly sampled population. J Clin Microbiol 1994; **32**: 323–30.
12. Stuart JM, Cartwright KAV, Robinson PM, Noah ND. Effect of smoking on meningococcal carriage. Lancet 1989; ii: 723–5.
13. Thomas JC, Bendana NS, Waterman SH, et al. Risk factors for carriage of meningococcus in the Los Angeles County Men's jail system. Am J Epidemiol 1991; **133**: 286–95.
14. Kaiser AB, Hennekens CH, Saslaw MS, Hayes PS, Bennett JV. Sero-epidemiology and chemoprophylaxis of disease due to sulphonamide-resistant *Neisseria meningitidis* in a civilian population. J Infect Dis 1974; **130**: 217–24.
15. De Wals P, Gilquin C, De Maeyer S, et al. Longitudinal study of asymptomatic meningococcal carriage in two Belgian populations of schoolchildren. J Infect 1983; **6**: 147–56.

16. Blakebrough IS, Greenwood BM, Whittle HC, Bradley AK, Gilles HM. The epidemiology of infections due to *Neisseria meningitidis* and *Neisseria lactamica* in a northern Nigerian community. J Infect Dis 1982; **146**: 626–37.
17. Olcén P, Kjellander J, Danielsson D, Lindquist BL. Epidemiology of *Neisseria meningitidis*: prevalence and symptom from the upper respiratory tract in family members to patients with meningococcal disease. Scand J Infect Dis 1981; **13**: 105–9.
18. Filice GA, Hayes PS, Counts GW, Griffiss JM, Fraser DW. Risk of group A meningococcal disease: bacterial interference and cross-reactive bacteria among mucosal flora. J Clin Microbiol 1985; **22**: 152–6.
19. Block C, Raz R, Frasch CE, et al. Re-emergence of meningococcal carriage on three-year follow-up of a kibbutz population after whole-community chemoprophylaxis. Eur J Clin Microbiol Infect Dis 1993; **12**: 505–11.
20. Caugant D, Høiby EA, Rosenqvist E, Frøholm LO, Selander RK. Transmission of *Neisseria meningitidis* among asymptomatic military recruits and antibody analysis. Epidemiol Infect 1992; **109**: 241–53.
21. Hirsch A. Epidemic cerebro-spinal meningitis. In: Handbook of geographical and historical pathology. Vol. III. Diseases of organs and parts (translated from the German by Creighton C.). London: New Sydenham Society, 1886: 547–94.
22. Glover JA. Observations of the meningococcus carrier rate and their application to the prevention of cerebrospinal fever. Special Report series of the Medical Research Council (London) 1920; **50**: 133–65.
23. Dudley SF, Brennan JR. High and persistent carrier rates of *Neisseria meningitidis* unaccompanied by cases of meningitis. J Hyg 1934; **34**: 525–41.
24. Wenzel RP, Davies JA, Mitzel JR, Beam WE. Non-usefulness of meningococcal carriage rates. Lancet 1973; ii: 205.
25. Olcén P, Kjellander J, Danielsson D, Lingquist BL. Culture diagnosis of meningococcal carriers: yield from different sites and influence of storage in transport medium. J Clin Pathol 1979; **32**: 1222–5.
26. Eldridge J, Sutcliffe EM, Abbott JD. Serological grouping of meningococci and detection of antigen in cerebrospinal fluid by co-agglutination. Med Lab Sci 1978; **35**: 63–6.
27. Abdillahi H, Poolman JT. *Neisseria meningitidis* group B serosubtyping using monoclonal antibodies in whole-cell ELISA. Microb Pathog 1988; **4**: 27–32.
28. Crowe CC, Sanders WE, Longley S. Bacterial interference. II. Role of the normal throat flora in prevention of colonisation by group A streptococci. J Infect Dis 1973; **128**: 527–32.
29. Jones DM, Kaczmarski EB. Meningococcal infections in England and Wales: 1993. CDR Rev 1994; **4**: R97–100.
30. Francis B, Green M, Payne C. The Glim system: release 4 manual. Oxford: Oxford University Press, 1993.
31. Computing Resource Center. Stata reference manual: release 3, vol. 2, 5th ed. Santa Monica, CA, USA, 1992.
32. OPCS. Living in Britain: results from the 1994 General Household Survey. London: HMSO, 1996.
33. Blackwell CC, Tzanakaki G, Kremastinou J, et al. Factors affecting carriage of *Neisseria meningitidis* among Greek military recruits. Epidemiol Infect 1992; **108**: 441–8.
34. Broome CV. The carrier state: *Neisseria meningitidis*. J Antimicrob Chemother 1986; **18** (Suppl A): 25–34.
35. Pether JVS, Scott RJD, Hancock P. Do meningococci cause sore throats? Lancet 1994; **344**: 1636.
36. Jones DM, Kaczmarski EB. Meningococcal infections in England and Wales: 1991. CDR Rev 1992; **2**: R61–3.
37. Jones DM, Sutcliffe EM. Group A meningococcal disease in England associated with the Haj. J Infect 1990; **21**: 21–5.
38. Rolleston H. Lumleian lectures on cerebro-spinal fever. Lecture 1. Lancet 1919; i: 541–9.
39. Phair JJ. Meningococcal meningitis. In: Coates JB, Hoff EC, Hoff PM, eds. Preventive medicine in World War II, vol. IV. Communicable diseases transmitted chiefly through respiratory and alimentary tracts. Washington, DC: Office of the Surgeon General, Department of the Army, 1958: 191–209.
40. Stanwell-Smith RE, Stuart JM, Hughes AO, Robinson P, Griffin MB, Cartwright K. Smoking, the environment and meningococcal disease: a case-control study. Epidemiol Infect 1994; **112**: 315–28.
41. Fischer M, Hedberg K, Cardosi P, et al. Tobacco smoke as a risk factor for meningococcal disease. Pediatr Infect Dis J 1997; **16**: 979–83.
42. Cooke RPD, Riordan T, Jones DM, Painter MJ. Secondary cases of meningococcal infection among household contacts in England and Wales, 1984–7. BMJ 1989; **298**: 555–8.