

Factors affecting carriage of *Neisseria meningitidis* among Greek military recruits

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SUMMARY

Greek military recruits (993) were examined for carriage of meningococci during July 1990. Blood, saliva and throat swab specimens were obtained and each recruit answered a questionnaire providing information on age, education (a measure of socioeconomic level), place of residence, smoking habits and recent infections.

The overall carriage rate was 25% but differed between the two camps: 79/432 (18%) in Camp A and 168/561 (30%) in Camp B ($P < 0.0005$). In Camp B, there were significantly higher proportions of recruits who were non-secretors ($P < 0.0005$), and/or heavy smokers ($P < 0.0005$). They were also younger (< 19 years old) ($P < 0.001$), and on the whole had fewer years of education ($P < 0.0005$). By univariate analysis, carriage was significantly associated with smoking. By multiple logistic regression analysis, carriage was associated with smoking ($P < 0.001$), age ($P < 0.01$) and the camp in which the recruits were based ($P < 0.01$). Among recruits in Camp B, 15/38 (40%) of those with recent viral infections were carriers compared with 30% for the camp in general.

INTRODUCTION

Following the epidemic of meningitis during the 1960s, the incidence of meningococcal disease in Greece declined steadily until 1988. Since 1988, the number of cases reported to the Ministry of Health has increased; in the first 6 months of 1990, there were 102 cases compared with 87 for all of 1989 (Table 1). This apparent increase in disease due to *Neisseria meningitidis* prompted the present work as the last study of meningococcal carriage in a Greek population was carried out before elucidation of some of the genetic and environmental factors affecting carriage [1].

Epidemiological studies have shown that non-secretors, individuals incapable of secreting the glycoprotein form of their ABO blood group antigens, are significantly over-represented among patients with meningococcal disease [2, 3]

Table 1. *Incidence of meningococcal disease in Greece*

Year	No. cases	Deaths no. (%)
1968	1064	48 (4.5)
1973	765	39 (5.1)
1978	286	18 (6.3)
1983	102	9 (8.8)
1985	105	6 (5.7)
1986	88	7 (7.9)
1987	85	4 (4.7)
1988	76	2 (2.6)
1989	87	6 (6.9)
1990 (1st 6 months)	102	

and among carriers in a school population [4]. Prolonged outbreaks have occurred in countries such as Iceland and in areas of Britain [5, 6] where the proportion of non-secretors is significantly higher than the proportion (20–25%) predicted for European populations [7].

Environmental factors have also been suggested as playing a role in susceptibility to meningococcal disease [8, 9] or carriage of the bacteria. Smoking or passive exposure to cigarette smoke has been associated with isolation of *Neisseria meningitidis* from healthy asymptomatic adults and teenagers [4, 10]. Virus infections have been suggested as predisposing factors for susceptibility to meningococcal disease [11] and to carriage of potentially pathogenic bacteria [12].

In the study reported here we wished to determine: (1) if isolation of meningococci was associated with non-secretion in the populations examined; (2) if smoking was associated with carriage in a closed population; (3) if an upper respiratory tract or other infection might be a factor contributing to carriage of meningococci; (4) if there might be geographical regions in Greece in which there are higher proportions of non-secretors and if the prevalence of meningococcal disease was increased in these areas.

SUBJECTS AND METHODS

Greek military recruits (993) from two camps, one at Athens and another at Avlona, were examined for carriage of meningococci during the third week of July 1990. Ethical permission for the study was granted by the Ministry of Defence. An explanation of the purpose of the study and the specimens required was part of the questionnaire and each subject signed his consent to participate in the study. The recruits came from all regions of Greece except the Ionian islands, and had been in the camps from 3 to 5 days at the time of the study. Throat swabs, blood and saliva specimens were obtained. Throat swabbing was carried out by the same four members of the team on both occasions (C.C.B., G.T., A.M., D.M.W.). Blood was not obtained from a number of recruits who fainted because of the unusually high temperature (41 °C). Each recruit filled in a questionnaire providing information on age, occupation, education level, places of residence and birth, smoking habits, and recent infections. A member of the research team checked the form to ensure all categories had been completed and to answer any questions. This information

and results of laboratory examinations were coded for confidentiality and stored in a Database 3 plus data base. The information on the data base was recalled and checked with the original questionnaire.

ABO blood group was determined by slide agglutination with monoclonal anti-A and anti-B antibodies (Scottish National Blood Transfusion Service); Lewis blood group was determined by tube agglutination with monoclonal anti-Lewis^a (Le^a) and anti-Lewis^b antibodies (Scottish National Blood Transfusion Service). Non-secretors can express only Le^a, but secretors express Le^b. The blood specimens were centrifuged on the day of collection and the plasma stored at -20°C .

Throat swabs were plated directly onto modified New York City medium [13] and incubated for 48 h. Colonies were examined for oxidase production, Gram stain and carbohydrate utilization.

Two-way frequency tables were analysed by χ^2 tests with Yates' correction in the case of 2×2 tables. Multiple logistical regression was used to examine the association between carriage rates and other factors adjusted for each other.

RESULTS

Isolation of N. meningitidis

N. meningitidis was isolated from 247 (25%) of the 993 recruits examined. The carriage rate differed significantly between the two camps. There were 79 isolates obtained from 432 recruits (18%) in the camp at Athens compared with 168 from the 561 (30%) at Avlona ($\chi^2 = 17.54$; $P < 0.0005$).

Characteristics of the two populations

Distribution of ABO and Lewis blood group phenotypes

There was no difference in the distribution of the ABO blood groups between the two camps. Among the recruits who expressed Lewis antigen, there was a significantly higher proportion of Le^a/non-secretors in the camp at Avlona, 140/534 (26%), compared with that in the camp at Athens, 60/390 (15%) ($\chi^2 = 14.96$, $P < 0.0005$).

Age and education

The ages of the two populations differed significantly. The median age was 19 for both camps; however, there was a higher proportion of recruits under 19 in the camp at Avlona (4%) than in that at Athens (1%) ($\chi^2 = 12.96$, $P < 0.001$).

There was also a significant difference in the level of education of the recruits in the two camps, particularly at the two extremes. Among the recruits at Avlona 14% had only 6 years of schooling compared with 7% among those at Athens; and, only 2% of the recruits at Avlona had attended university while 7% of those at Athens had done so ($\chi^2 = 30.32$, $df = 4$, $P < 0.0005$).

Smoking

There was no significant difference between the proportion of smokers in the Athens camp (62%) and in Avlona (68%); but there were significantly more heavy smokers (> 30 cigarettes a day) in Avlona ($\chi^2 = 27.64$, $df = 4$, $P < 0.0005$) (Table 2).

Table 2. *Cigarette consumption in the two recruit groups*

No. cigarettes/day	Athens (<i>n</i> = 431)	Avlona (<i>n</i> = 560)
	No. (%)	No. (%)
0	166 (38)	182 (32)
1-10	53 (12)	63 (11)
11-20	127 (29)	133 (24)
21-30	62 (14)	96 (17)
> 30	23 (5)	86 (15)

Table 3. *Carriage rates and cigarette consumption*

No. cigarettes/ day	Total	Carriers no. (%)	Non-carriers no. (%)
0	355	56 (16)	299 (84)
1-10	116	29 (25)	87 (75)
11-20	260	66 (25)	194 (75)
21-30	157	44 (28)	113 (72)
> 30	109	40 (37)	69 (63)

Prevalence of other infectious diseases

Only 59 of the recruits reported symptoms of upper respiratory tract infections (URTI) within the previous 2 weeks, 21 of the 432 at Athens (4.9%) and 38 of the 561 (6.7%) at Avlona.

Factors associated with carriage of meningococci

There was no difference in the proportions of carriers among the four ABO blood groups. The proportion of carriers among Le^a/non-secretors (22% at Athens, 32% at Avlona) was higher than that among Le^b/secretors (17% at Athens, 30% at Avlona), but the difference was not significant.

The proportion of carriers was significantly higher among smokers and was greatest among those smoking more than 30 cigarettes per day (Table 3) ($\chi^2 = 23.19$, *df* = 4, $P < 0.0005$). Although the proportion of carriers was significantly increased among smokers in both camps (Athens, $\chi^2 = 5.42$, *df* = 1, $P < 0.05$; Avlona $\chi^2 = 9.05$, *df* = 1, $P < 0.01$), when the data for carriers were analysed by χ^2 test, there was no significant difference between the two camps associated with numbers of cigarettes per day ($\chi^2 = 9.482$, *df* = 4, $P > 0.05$) (Table 4).

Meningococci were isolated from 17 (29%) of the 59 recruits who had symptoms of URTI, 2 of the 21 (9.5%) at Athens and 15 of the 38 (39.5%) at Avlona. Among the 15 at Avlona from whom meningococci were isolated, 6 (40%) were Le^a/non-secretors compared with 4 (20%) Le^a/non-secretors among the 20 with recent symptoms of URTI but from whom no meningococci were isolated. The numbers of recruits with symptoms of URTI were too small for statistical analysis.

Multiple logistic regression analysis identified three main factors which were significantly associated with carriage: smoking ($P < 0.001$); age less than 19 years ($P < 0.01$) and the camp in which the recruits were based ($P < 0.01$). Various

Table 4. *Smoking patterns and carriage of N. meningitidis in the two camps*

No. cigarettes/ day	Athens (n = 431)		Avlona (n = 560)	
	Total No. (%)	Carriers No. (%)	Total No. (%)	Carriers No. (%)
0	166 (38)	20 (27)	182 (32)	36 (22.5)
1-10	53 (12)	10 (13)	63 (11)	19 (12)
11-20	127 (29)	26 (35)	133 (24)	40 (25)
21-30	62 (14)	15 (20)	96 (17)	29 (18)
> 30	23 (5)	4 (15)	86 (15)	36 (22.5)
Total	431	75	560	160

combinations of categories were tested for difference in carriage rate when adjusted for these three factors but no other variables were found to be significant.

Regional variations in ABO and Lewis blood group antigens

There was no difference in the distribution of the ABO blood groups in the two major population centres, Athens and Eastern Macedonia. There was, however, a significantly higher proportion of Le^a/non-secretors, 54/183 (29.5%) among the recruits from Eastern Macedonia compared with those from Athens, 35/235 (15%) ($\chi^2 = 12.25, P < 0.0005$).

DISCUSSION

As expected, compared with carriage rates reported for civilian populations (5-8% for Greek secondary school children), there was a higher proportion of carriers (25%) among the recruits examined in this study. This figure is similar to that found for a British military establishment (23%) [14] but slightly lower than that found in the 1970s for Greek military recruits (33%) [1]. The significant difference in the proportion of carriers between the two camps, 18% at Athens compared with 30% at Avlona ($P < 0.0005$), was not anticipated.

Smoking [4, 10], passive exposure to cigarette smoke [10], secretor status [4] and age [15] have all been reported to be associated with carriage of meningococci. The distribution of the ABO blood group phenotypes did not differ between the two camps and were similar to those we found in a previous study [16] and that reported by Hirzfeld and Hirzfeld in 1919 [17]. Several of the factors examined in the study varied between the two camps. Compared with the recruits at Athens, among those at Avlona where there was a significantly higher proportion of carriers of meningococci, there were significantly more heavy smokers, non-secretors, younger recruits (less than 19 years old), and recruits of lower socioeconomic groups as assessed by years of education.

Smoking was the factor most strongly associated with carriage in both univariate and multivariate analyses. It has been suggested that the higher proportion of smokers and heavy smokers among military personnel [18] might contribute to the increased prevalence of meningococcal disease and the higher rates of carriage among military recruits. The proportions of smokers in the two camps, 62 and 68%, were higher than the 47% found in a previous study among Greek male patients referred for gastroscopy [15]. In Britain smoking is associated

with socioeconomic status; unskilled individuals are more likely to smoke than those in professional groups [19]. A similar pattern was observed among the recruits; those with fewer years of education were more likely to be smokers.

In a previous study of teachers and pupils in a Scottish secondary school following an outbreak of meningitis, we found an association between non-secretion and carriage of meningococci [4]. In the present study, in both camps, the proportions of non-secretors were increased among carriers; but, these differences were not significant compared with the secretors among carriers. Assessment of both host and parasite characteristics might elucidate the differences in association with secretor status. The majority (35.5%) of serotypable strains among the 121 isolates in the Scottish study were serotype 4; and 55% of the carriers of this serotype were non-secretors. Only 9 (1.8%) isolates in the present study were serotype 4, but 37.5% of the carriers were non-secretors. The proportion of non-secretors among carriers of serotype 14 isolates was 25 and 22% for the Scottish and Greek populations. A similar pattern was observed for non-serotypable isolates.

The multiple logistic regression analysis identified age (those less than 19 years of age) to be a significant variable ($P < 0.01$). This agrees with the findings for carriers in the Stonhouse survey. The highest proportion of carriers was found in the 15–19 year age group [15].

There is evidence that viral infections can predispose individuals to carriage of potentially pathogenic bacteria [12]; and binding of streptococci and staphylococci to cell surfaces is enhanced in experimental models examining infection with influenza virus [20, 21]. The present study was carried out in July when there is a low prevalence of upper respiratory viral infections; however, of the 59 recruits with symptoms of virus infection, 29% were carriers. This was particularly apparent among recruits at Avlona where the carriage rate was 30% overall but nearly 40% among those with virus infections. In a recent study, a higher proportion of patients with meningococcal disease had a history of flu-like illness than age- and sex-matched controls; however, the differences were not statistically significant [22]. A second study of recruits was undertaken in January 1991 to assess carriage when viral infections are more prevalent; the analysis of this study is not complete at present.

In two areas of Britain where there have been prolonged outbreaks of meningococcal disease, the proportions of non-secretors have been reported to be higher than the 20–25% predicted for European populations [5, 6]. In this study we found a significantly higher proportion of recruits from Eastern Macedonia were non-secretors (29.5%) compared with the other major centre of population, Athens (15.3%). As there are anecdotal reports that meningococcal disease is more prevalent in Macedonia, the numbers of cases reported to the Ministry of Health from these two areas is now being closely monitored.

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