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The Stonehouse study: secretor status and carriage of *Neisseria* species

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SUMMARY

The genetically determined inability to secrete the water-soluble glycoprotein form of the ABO blood group antigens into saliva and other body fluids is a recognized risk factor for meningococcal disease. During a community-wide investigation of a prolonged outbreak of disease due to a B15:P1.16 sulphonamide-resistant strain of *Neisseria meningitidis* in Stonehouse, Gloucestershire (the Stonehouse survey), the ABO blood group and secretor status of almost 5000 residents was determined.

The proportion of non-secretors in the Stonehouse population was significantly higher than the proportion of non-secretors among blood donors in the South West Region and in England generally. Seven of 13 Stonehouse residents with meningococcal disease who were tested were found to be non-secretors, a high proportion. The outbreak in Stonehouse cannot be explained solely in terms of the increased proportion of non-secretors. There was no clear correlation between the proportions of non-secretors in different areas within the town and the incidence of cases of meningococcal disease.

Carriers of meningococci, whether outbreak or other strains, were not more likely to be non-secretors. The reasons why non-secretors are more susceptible to meningococcal disease remain to be determined, but they do not appear to be related to carriage of meningococci.

INTRODUCTION

The large-scale survey undertaken in Stonehouse, Gloucestershire provided an opportunity to examine factors that might explain the prolonged local outbreak of disease due to serogroup B, serotype 15 P1.16, sulphonamide-resistant (B15 P1.16R) *Neisseria meningitidis* (Stuart *et al.* 1987). The results of the bacterial screening established that there was a low carriage rate of the B15 P1.16R strain in a defined community at a time of high disease activity. An outbreak continuing for several years implies that a virulent strain is moving slowly through a susceptible community. The factors responsible for the apparent virulence of the outbreak strain remain to be elucidated (Cartwright *et al.* 1987).

Environmental influences contributing to susceptibility of the host to these infections include severe overcrowding (Kaiser *et al.* 1974) and passive smoking

(Haneberg *et al.* 1983; Stuart *et al.* 1988). The former does not appear to play a significant role in the Stonehouse outbreak. Cigarette consumption is higher among men and women in unskilled jobs compared with those in professional employment (Wald *et al.* 1988), and there was a higher proportion of household heads with manual occupations in the two areas of the town with 14 of the 15 cases (Stuart *et al.* 1987).

We have identified a genetic character that is found in a significant proportion of patients with bacterial meningitis compared with individuals within their respective local control populations. This is the inability of an individual to secrete the water-soluble glycoprotein form of ABO blood group antigens (Blackwell *et al.* 1986*a*, *b*). The secretor gene is inherited in a Mendelian-dominant pattern; in most west European populations 20–25% express the recessive non-secretor phenotype associated with increased susceptibility to meningococcal infections (Eriksson *et al.* 1986; Race & Sanger, 1975). In areas of the world in which there have been prolonged outbreaks or epidemics of meningococcal disease such as Iceland and northern Nigeria and secretor status has been investigated, the proportions of non-secretors in these populations are higher. In Iceland non-secretors make up about 40% of the population (Thordarson *et al.* 1972), and near Maiduguri, Nigeria almost 50% (Blackwell *et al.* 1988). One objective of the survey was, therefore, to determine the proportion of non-secretors in Stonehouse.

There is a significantly higher proportion of non-secretors among patients with rheumatic fever and also a higher proportion of non-secretors among carriers of group A *Streptococcus pyogenes* (Haverkorn & Gosling, 1969). The increased susceptibility of non-secretors to meningococcal disease might be due to an increased tendency to acquire the outbreak strain compared with secretors. The second objective was to establish the proportion of non-secretors amongst carriers of meningococci, especially those carrying the outbreak strain.

SUBJECTS AND METHODS

The population examined has been described in detail (Stuart *et al.* 1987). ABO blood groups were determined for Stonehouse residents who participated in the study and from whom a blood sample (EDTA anti-coagulant tube) was collected. Because contamination of saliva with blood can result in a false positive secretor result when tested by the haemagglutination inhibition method, every eighth specimen was tested for Lewis blood group. Secretors express Lewis^b on their erythrocytes and non-secretors Lewis^a. Generally, 3–4% of individuals express neither Lewis^a nor Lewis^b on their erythrocytes; the majority of these are secretors by the saliva test. ABO group was determined by slide agglutination with monoclonal anti-A and anti-B antibodies supplied by the Edinburgh and South East Scotland Blood Transfusion Service. Lewis groups were tested by tube agglutination with polyclonal anti-Lewis^a and anti-Lewis^b antisera (Dade). All blood specimens were processed within 24 h of collection.

Saliva specimens were frozen within 3 h of collection and transported to Edinburgh for determination of secretor status by the haemagglutination inhibition method (Mollison, 1979) with monoclonal anti-A and anti-B antibodies and *Ulex europaeus* lectin (Sigma) for detection of H antigen (the antigen of blood

group O). Isolation and identification of the *Neisseria* species from the Stonehouse residents has been reported (Cartwright *et al.* 1987).

The results were analysed by the data processing system described previously (Stuart *et al.* 1987).

The Lewis blood groups of 672 randomly selected donors from the area served by the South Western Region Blood Transfusion Service were determined by Dr I. D. Fraser and colleagues (South Western Region Blood Transfusion Service).

RESULTS

ABO blood groups of Stonehouse residents

The distribution of the ABO blood groups among 4899 Stonehouse residents tested is similar to that reported for England (Mourant *et al.* 1976): 2201 group A (44·9%); 411 group B (8·4%); 2147 group O (43·8%); 140 group AB (2·9%).

Secretor status and Lewis blood groups of Stonehouse residents

Saliva specimens were obtained from 4906 (98%) of Stonehouse residents who participated in the study (Table 1). Both the saliva tests and the Lewis blood groups indicate there is a higher proportion of non-secretors in Stonehouse than the predicted 20–25%. By the saliva test, 3554 (72·4%) were secretors and 1352 (27·6%) were non-secretors (Table 2). Of the 15 Stonehouse residents who had had meningococcal infection we examined saliva specimens from 13; 7 of the 13 (54%) were non-secretors.

Of the 672 blood donors from the South Western Regions, 40 (5·9%) were Lewis negative and 632 were Lewis positive; of the remainder 484 (76·5%) were Lewis^b and 148 (23·4%) were Lewis^a. Among the 598 blood specimens from the Stonehouse study tested, 32 (5·3%) were Lewis negative; 7 (22%) were non-secretors and 25 (78%) were secretors by the saliva test. There was no saliva specimens from the blood donors for comparison with those from the study group; therefore, the Lewis negative individuals of both groups were not included in the analysis.

The difference between the distribution of Lewis groups among Stonehouse residents, 276 Lewis^b (67·3%) and 134 Lewis^a (32·7%), and the population in the South Western Region is highly significant ($\chi^2 = 10{\cdot}2$, D.F. $= 1$, $P < 0{\cdot}005$). The difference between the distribution of Lewis groups among non-Stonehouse residents in the study (e.g. children attending school in Stonehouse), 112 Lewis^b (72%) and 44 Lewis^a (28%), and the South Western Region was not statistically significant $\chi^2 = 1{\cdot}3$, D.F. $= 1$, $P > 0{\cdot}05$) (Table 3).

Saliva from Lewis^a individuals (non-secretors) may give false positive secretor results if it is contaminated with small amounts of blood as a result of poor oral hygiene or, conversely, over-vigorous brushing of teeth. There was, however, close agreement between the results of the saliva tests and the Lewis blood groups. Among both residents and non-residents tested 389 were Lewis^b and 371 (95·4%) were secretors. The discrepancy might be due to weak expression of the secretor gene or to dilution of very small saliva specimens. Of the 178 Lewis^a individuals, 165 (92·7%) were non-secretors by the saliva test.

Table 1. *Specimens tested from participants in Stonehouse survey for secretor state and Lewis blood groups by area of residence*

	Population	Participants		Saliva		Lewis+ blood specimens	Participants (%)
		No.	(%)	No.	(%)		
Park Estate	1729	1273	(73·6)	1264	(99·3)	119	(9·4)
Verney Fields	1836	1216	(66·2)	1184	(97·4)	103	(8·7)
Little Australia	869	750	(86·3)	738	(98·4)	61	(8·3)
Rosedale	1423	1226	(86·2)	1188	(96·9)	102	(8·6)
Bridgend	778	541	(69·5)	532	(98·3)	25	(4·6)
Total	6635	5006	(75·4)	4906	(98)	410	(8·4)

Secretor status and Lewis blood group distribution by area of residence in Stonehouse

The numbers of specimens tested for secretor status and Lewis group for the five areas of Stonehouse are shown in Table 1. Table 2 compares the secretor status and Lewis blood group distributions by area of residence in Stonehouse. While there is close agreement between the saliva tests and Lewis groups for Rosedale and Bridgend areas, the results suggest that the proportion of non-secretors in Park Estate, Little Australia and Verney Fields is underestimated by the saliva test. Except for Rosedale, the proportion of Lewis[a] individuals was over 30%; Park Estate-33%, Verney Fields-36%, Little Australia-38% and Bridgend-33%. There was no significant difference in the proportion of Lewis[a] individuals.

When secretor status was analysed by age bands, the highest proportion of non-secretors was found in the 0–4 and 5–9 age groups (Table 4).

Distribution of secretors and non-secretors in Stonehouse schools

By the saliva test there were no significant differences in the proportions of secretors and non-secretors attending state or private schools in Stonehouse, although most children attending private schools were not Stonehouse residents. The number of pupils for whom Lewis blood group was determined was small, but the results suggest that the numbers of non-secretors were underestimated, particularly among those at the state schools.

Among the schoolchildren tested for Lewis group the proportion of Lewis[a] individuals among the 122 from state junior and state secondary schools was 37% compared with 28% among 75 pupils from the corresponding private schools; but the difference was not statistically significant.

Carriage of Neisseria *species and secretor status*

Among the 69 Stonehouse residents who were carriers of the outbreak strain we examined saliva specimens from 67; there were 47 secretors (70%) and 20 non-secretors (30%). Among the carriers of the non-outbreak strains, 327 (71%) were secretors and 132 (29%) were non-secretors (Table 5).

Tables 6 and 7 include data for carriers resident in Stonehouse as well as non-residents. There were no significant differences in the proportion of non-secretors among the carriers analysed by serogroup (Table 6). There were no differences in the proportion of non-secretors carrying serotypable strains (31%) compared with those carrying non-serotypable strains (31%) (Table 7).

Table 2. *Secretor state and Lewis blood group distributions by area of residence in Stonehouse*

Area	Saliva samples (No.)	Secretors		Non-secretors		Le$^+$ specimens (No.)	Le^b		Le^a		No.* cases
		No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Park Estate	1264	917	(72·5)	347	(27·5)	119	80	(67)	39	(33)	11
Verney Fields	1836	862	(72·8)	322	(27·2)	103	66	(64)	37	(36)	3
Little Australia	738	556	(75·3)	182	(24·7)	61	38	(62)	23	(38)	0
Rosedale	1188	864	(72·7)	324	(27·2)	102	75	(73·5)	27	(26·5)	1
Bridgend	532	355	(66·7)	177	(33·3)	25	17	(69)	8	(32)	0
Total	4906	3554	(72·4)	1352	(27·6)	410	276	(67·3)	134	(32·7)	15

* The cases in each of the areas are included in the figures for the residents.

Table 3. *Secretor state of Stonehouse residents compared with UK as a whole*

	Secretors		Non-secretors			
	No.	(%)	No.	(%)	χ^2	*P*
Saliva test						
Stonehouse	3554	(72·5)	1352	(27·6)		
England (pooled data,* 10 studies)	5747	(76)	1819	(24)	18·4	< 0·0005
Lewis blood group						
Stonehouse	277	(67·3)	134	(32·7)		
England	835	(77·2)	246	(22·8)	14·7	< 0·0005
South West Region	484	(76·6)	148	(23·4)	10·2	< 0·005

* Mourant, Kopec, & Domaniewska (1976).

Table 4. *Secretor state and number of cases in Stonehouse by age group*

		Secretors		Non-secretors		
Age	Number	No.	(%)	No.	(%)	No. cases
0–4	296	199	(67·2)	97	(32·8)	2
5–9	434	299	(68·9)	135	(31·1)	7
10–14	362	275	(76)	87	(24)	4
15–19	384	276	(71·9)	108	(28·1)	0
20–24	308	278	(73·2)	102	(26·8)	0
25–34	807	568	(70·4)	239	(29·6)	1
35–44	674	487	(72·3)	187	(27·7)	0
45–54	439	325	(74)	114	(26)	1
55–64	507	366	(72·2)	141	(27·8)	0
65+	647	462	(71·4)	185	(28·6)	0
Total	4930	3535	(71·7)	1395	(28·3)	15

Table 5. *Distribution of secretors and non-secretors among carriers of meningococci in Stonehouse*

	Secretor		Non-secretor		
Organism	No.	(%)	No.	(%)	Total
Outbreak strains	47	(70)	20	(30)	67
Non-outbreak strains	327	(71)	132	(29)	459

Carriage of *Neisseria lactamica* among Stonehouse residents was analysed with regard to age and secretor status (Table 8). The proportion of non-secretors among the carriers was similar to the proportion of non-secretors in the age band.

DISCUSSION

There is a significantly higher proportion of individuals with Lewis[a] blood group in Stonehouse compared with blood donor controls from the South Western Region and other English populations studied (Table 3). Although the saliva test underestimates the numbers of non-secretors, comparison of the results with data

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Table 6. *Distribution of secretors and non-secretors among carriers of serogroupable and non-serogroupable* N. meningitidis

		Secretors		Non-secretors	
Serogroup	Total	No.	(%)	No.	(%)
Serogroupable		262	(72)	104	(28)
A	3	3		—	
B	211	151	(72)	60	(28)
C	32	23	(72)	9	(28)
W135	22	15	(68)	7	(32)
X	4	4		0	
Y	53	35	(66)	18	(34)
Z	29	22	(76)	7	(24)
Z/29E	19	13	(68)	6	(32)
29E	12	9	(75)	3	(25)
Non-serogroupable	309	209	(68)	100	(32)

Table 7. *Distribution of secretors and non-secretors among carriers analysed by serotype of* N. meningitidis

		Secretors		Non-secretors	
	Total	No.	(%)	No.	(%)
Serotypable	288	199	(69)	89	(31)
Serotypes				—	
2a		4			
2b		6		1	
2c		1		—	
P1.16	25	13	(52)	12	(48)
P1.2	77	52	(68)	25	(32)
P1.3	33	27	(82)	6	(18)
15	67	41	(61)	26	(39)
15 P1.16	74	55	(74)	19	(26)
Non-typable	260	180	(69)	80	(31)

from 10 pooled studies of English populations in which saliva was tested for ABH secretion (Mourant *et al.* 1976) shows a higher proportion of non-secretors in Stonehouse.

The relative risk of non-secretors developing meningococcal disease in Stonehouse was 2·4 (95% confidence interval 1·3 to 4·3). This is similar to the relative risk calculated from previous studies in Iceland (1·7), Nigeria (2·9) and Scotland (6·2) (Blackwell *et al.* 1988). If there had been 23% (the South Western Region percentage) instead of 33% non-secretors in Stonehouse with the same attack rate and the same relative risk (2·4), the number of cases would have been 13·6 instead of the 15 observed.

Non-secretors appeared to be more likely to be cases within Park Estate than secretors. In Park Estate 33% of the residents tested were Lewis[a] compared with 5 of the 8 cases (62·5%) tested. In Verney Fields 36% of the residents were Lewis[a] compared with 2 of the 5 cases (40%). These figures indicate a trend, but are too small for statistical analysis.

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Table 8. *Secretor state and age of* N. lactamica *carriers in Stonehouse*

Age	No. isolates	Secretors No.	Secretors (%)	Non-secretors No.	Non-secretors (%)	Non-secretors in Stonehouse (%)
0–4	46	26	(62)	16	(38)	32·8
5–9	20	14	(70)	6	(30)	31·1
10–14	11	6	(55)	5	(45)	24
15–19	11	7	(64)	4	(36)	28·1
20–24	10	7	(70)	3	(30)	26·8
25–34	27	19	(73)	7	(27)	29·6
35–44	7	7		0		27·7
45–54	6	6		0		26
55–64	5	4		1		27·8
65+	4	2		2		28·6
Total	147	98	(69)	44	(31)	

These results suggest that while the proportion of non-secretors who are more susceptible to bacterial meningitis is significantly higher in Stonehouse, it is not sufficient of itself to explain the outbreak.

Our suggestion that blood contamination might account for differences between the saliva tests and Lewis blood groups is supported by detection of Lewis^a antigen in the saliva of a number of 'secretors' by a recently developed ELISA with monoclonal antibodies (unpublished observations). The higher proportion of non-secretors in the under-10 age group might reflect less periodontal disease. In developed countries, periodontal disease is uncommon before puberty, but the frequency of disease begins to rise in the circumpubertal period (11–14 years) (Murray, 1983).

Blood contamination of the saliva could be due to inadequate oral hygiene which is often associated with poor economic background (Waerbaug, 1971; Murray, 1983). There were 21 pairs of blood and saliva specimens from Stonehouse residents in which there was disagreement between the results of the saliva test and the Lewis group. Of the 8 Lewis^a/secretor pairs (false secretors), 7 were from residents of Verney Fields and Park Estate where 14 of the 15 cases of meningitis occurred. The other was from a resident of Little Australia. Of the 13 Lewis^b/non-secretor specimens, 4 were from residents of Park Estate and Verney Fields and 9 from the other three areas.

Agreement between the saliva test and Lewis blood groups was better in areas where socioeconomic levels were higher – Rosedale and Bridgend (Table 3). In Park Estate and Verney Fields where most of the cases of meningococcal disease occurred, the proportion of non-secretors found by the saliva test is lower than that indicated by the proportion of Lewis^a individuals, and there was a higher proportion of household heads in semi-skilled/unskilled occupations. There were no cases of meningococcal disease in Little Australia which had the highest proportion of Lewis^a individuals (38%) but the lowest proportion of non-secretors by the saliva test (24·7%). In this area there was a higher proportion of household heads with skilled jobs than in Park Estate and Verney Fields. The sampling in Little Australia was not as random as in other areas of the town; over half (13/23) of the Lewis^a specimens were from residents in 3 of the 12 postcode areas.

Of the 61 blood specimens tested from Little Australia for Lewis group, there were only 3 in which the saliva test did not agree with the Lewis group; 1 was Lewis^a/secretor and 2 Lewis^b/non-secretor. The number of individuals in Little Australia tested for Lewis group was smaller, which might account for the differences observed for the two tests.

Factors associated with poorer socioeconomic background contribute to susceptibility to infectious diseases. Smoking which might be important in the transfer of meningococci from one individual to another is more prevalent among manual and unskilled workers (Wald *et al.* 1988).

The increased susceptibility of non-secretors to meningococcal infection is not associated with an increased carriage rate of the B15 P1.16 strain. As previous studies (Blackwell *et al.* 1988) have shown a higher proportion of non-secretors among patients with infection due to the three major serogroups of meningococci (serogroups B and C were predominantly isolated from the Icelandic and Scottish patients, serogroups other than B from the Nigerian patients), we did not expect to find an association between secretor status and carriage of any particular serogroups (Table 6). If the protein serotype antigens participate in lectin mediated attachment to carbohydrates on epithelial cells, the carbohydrate moieties of ABO or Lewis^b antigens present in the body fluids of secretors might bind to the bacteria reducing attachment of meningococci to epithelial cells. No consistent patterns was observed; the proportion of non-secretors was the same among carriers of either serotypable or non-serotypable strains.

The prolonged outbreak of disease due to the B15 P1.16 meningococcus in Stonehouse can be partly explained by the significantly higher proportion of non-secretors compared with the South Western Region in general. Our results do not parallel the model suggested for rheumatic fever in which a higher proportion of non-secretors was found among the carriers of group A streptococci (Haverkorn & Gosling, 1969). The increased susceptibility of non-secretors has yet to be explained but it does not appear to be due to increased carriage of these organisms.

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