

Carriage of *Neisseria* species in communities with different rates of meningococcal disease

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N LE SAUX, F ASHTON, M RAHMAN, et al. Carriage of *Neisseria* species in communities with different rates of meningococcal disease. *Can J Infect Dis* 1992;3(2):60-64. A single clone, *Neisseria meningitidis* serogroup C (C:2a:P1.2), was isolated from seven patients during a cluster of cases of meningococcal disease in Ontario in 1989. To determine whether the clone was present in asymptomatic individuals in the same population, pharyngeal swabs were taken from 7% (644 of 9125) of residents who were vaccinated during the outbreak. Rates of isolation of *Neisseria* species were also compared to those in two other geographical areas which did not have an elevated incidence of meningococcal disease. The rate of carriage of *N meningitidis* in the asymptomatic individuals sampled was between 1.9% and 5.4%. The clone isolated from patients was not present among the carrier strains as determined by sero- and subtyping and electrophoretic analysis of metabolic enzymes. Age greater than six years was the only factor associated with colonization with *N meningitidis*.

Key Words: Carriage, Clonality, Enzyme electrophoretic analysis, *Neisseria meningitidis*, Typing

Le portage des espèces de *Neisseria* dans les collectivités manifestant différents taux de méningite méningococcique

RESUME: Un clone unique de *Neisseria meningitidis* séro-groupe C (C:2a:P1.2) a été isolé chez sept patients lors d'une flambée de cas de méningite méningococcique en Ontario en 1989. Pour déterminer si le clone était présent chez les personnes asymptomatiques issues de la même population, on a effectué des prélèvements pharyngiens par écouvillonnage chez 7 % (644 sur 9,125) des résidents qui avaient été vaccinés à l'époque. Les taux d'isolement des espèces de *Neisseria* ont également été comparés à ceux de deux autres régions géographiques qui n'avaient pas une incidence élevée d'affections méningococciques. Le taux de portage de *N meningitidis* chez les sujets asymptomatiques étudiés se situait entre 1,9 et 5,4 %. Le typage et sous-typage sérologiques et l'analyse électrophorétique des enzymes métaboliques ont permis de déterminer que le clone isolé chez les patients ne se trouvait pas parmi les souches des porteurs. Un âge supérieur à six ans était le seul facteur associé à la colonisation par *N meningitidis*.

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THE PRESENCE OF *NEISSERIA MENINGITIDIS* IN THE UPPER respiratory tract is not usually associated with disease. The occasional acquisition of virulent strains of *N meningitidis* in this anatomical area is thought to be critical for the development of disease in susceptible individuals (1,2).

During the winter of 1988-89, an increase in the number of cases of meningococcal disease occurred in the province of Ontario (3). Within that period, nine clinical or culture-confirmed cases of meningococcal disease occurred in a predominantly rural county (Victoria County) comprising three townships and two towns surrounding Lindsay, Ontario. Census figures from 1986 estimated the total population of the area to be 28,135. The overall rate of meningococcal disease in the 'zero to 19 years' age group was 75 per 100,000; however, the rate was 198 per 100,000 in the '10 to 14 years' age group, and 50 per 100,000 in the 'five to nine years' age group. Of seven culture-confirmed cases, all had *N meningitidis* serogroup C, serotype 2a, subtype P1.2 (C:2a:P1.2) (4).

A study examining nasopharyngeal carriage of *Neisseria* species was undertaken to determine whether the outbreak strain of *N meningitidis* serogroup C was prevalent in the disease-free population of Victoria County. Two other control areas were sampled to determine whether carriage of the same clone was present in regions that did not have an elevated incidence of meningococcal disease. In the control areas, the authors also attempted to identify risk factors associated with carriage of *Neisseria* species.

SUBJECTS AND METHODS

The neisseria carriage study was done in Victoria County (the area with an elevated incidence of disease) and Perth County, Ontario and in three health units in southern Alberta (areas that did not have increased numbers of patients with meningococcal disease).

The culture survey was done during an immunization program with quadrivalent vaccine (A,C,Y,W-135) for persons aged six months to 18 years in the Victoria County town of Lindsay, Ontario in February 1989. Cultures were obtained just prior to receipt of meningococcal vaccine. A total of 644 of 9125 people (7%) who presented for vaccination had throat cultures done. Information on age, sex and treatment with rifampin in the two weeks prior to swabbing was obtained from subjects or their parents.

Perth County, surrounding Stratford, Ontario, is 270 km southwest of Victoria County. In this area, a convenience sample of schools was used, and children were selected using a table of random numbers from class lists provided by the schools. Questionnaires were sent home for completion. To select children below school age, the following two methods of recruitment were used. A random sample was taken of the 30% who attended licensed daycare facilities. For the 70% not

attending these facilities, recruitment involved children born after 1984 who presented to major or group medical practices in the area during the time swabbing took place. If the accompanying parent agreed, she or he completed the questionnaire and the child was swabbed.

The control area of the Lethbridge, Barons-Eureka-Warner and Chinook Health Units located in southern Alberta has similar demographics to the two Ontario areas and is located 3500 km west of Victoria County. Students in all schools in the three health units were randomly selected. Questionnaires and consent forms were mailed to the students' homes and returned to the school. Preschool children were selected randomly from routine immunization clinics in the health units where all preschool immunizations are done in Alberta.

Questionnaires and consent forms were completed at the immunization clinics. Swabbing in the control areas was completed within an eight week period. Exclusion criteria for participation was any current acute illness or fever.

Subjects in the two control areas were asked about age, sex, symptoms of respiratory illness and antibiotic use in the preceding two weeks, and history of serious or significant medical problems. Information was also obtained on family size, attendance at school, daycare, play groups, team sports and clubs, as well as parent education level.

The health units notified parents who had requested results of the culture. Parents were free to discuss results with their private physicians. Treatment of carriers was not recommended.

Laboratory methods: Swabbing consisted of rubbing the posterior pharynx behind the uvula with a cotton-tipped swab. In the two Ontario areas, swabs were inoculated directly onto New York City medium in JEMBEC plates. Carbon dioxide-generating tablets were added to the plates, which were then put in plastic bags. In Alberta, the swabs were inoculated onto plates with chocolate agar and modified Thayer-Martin medium and placed in Bio-Bag carbon dioxide transport systems (Marion Scientific, Missouri). Plates were incubated at 37°C within 2 h of swabbing using portable or local hospital incubators and examined after 24 to 48 h of incubation. Identification of *Neisseria* species, serogrouping, serotyping and subtyping were carried out as described previously (5-8). Monoclonal antibodies for sero- and subtyping were kindly supplied by Dr JT Poolman of the National Institute of Public Health and Environmental Protection in the Netherlands, and Dr WD Zollinger of the Walter Reed Army Institute in Washington, DC. Electrophoretic analysis of metabolic enzymes was carried out as described by Selander et al (9) and Caugant et al (10).

Data analysis: Data analysis was carried out using EPIINFO version 3 (Division of Disease Surveillance and Epidemiologic Studies, Centers for Disease Control,

TABLE 1
Distribution of *Neisseria* species present in selected asymptomatic populations

Geographical area	Negative*	<i>Neisseria meningitidis</i>	<i>Neisseria lactamica</i>	Other <i>Neisseria</i> species†	Overall carrier rate
Victoria County (n=644)	555 (86.2%)	35 (5.4%)	54 (8.4%)	0 (0%)	89 (13.8%)
Perth County (n=699)	590 (84.4%)	34 (4.9%)	72 (10.3%)	3 (0.4%)	109 (15.6%)
Alberta (n=773)	641 (82.9%)	15 (1.9%)	110 (14.2%)	7 (1.0%)	132 (17.1%)
Total (n=2116)	1786 (84.4%)	84 (4.0%)	236 (11.22%)	10 (0.5%)	330 (15.6%)

*No *Neisseria* species isolated. †Included five strains of *Neisseria perflava/sicca* and five strains of *Neisseria polysaccharea*

TABLE 2
Distribution of *Neisseria meningitidis* by serogroup in the selected asymptomatic populations

Area	Serogroup					Total
	B	C	Y	29e	NG	
Victoria County	10	5	8	0	12	35
Perth County	11	2	2	0	19	34
Alberta	4	0	3	2	6	15
Total	25	7	13	2	37	84
%	29.8	8.3	15.5	2.4	44.0	

NG Nongroupable

TABLE 3
Distribution of serosubtypes among serogroups of *Neisseria meningitidis* in the selected asymptomatic populations

Serosubtype	Serogroup					Total (%)
	B	C	Y	29e	NG	
2a:P1.2		3				3 (3.6)
2b:P1.-*	1					1 (1.2)
2b:P1.2		1				1 (1.2)
2c:P1.2			1			1 (1.2)
4:P1.-*	6				4	10 (12.0)
4:P1.1	3					3 (3.6)
4:P1.2				1	1	2 (2.4)
4:P1.7	1				1	2 (2.4)
4:P1.16	2			1	2	5 (6.0)
15:P1.-*	2	1	2		7	12 (14.5)
15:P1.7	1					1 (1.2)
NT:P1.-*	6	1	4		8	19 (22.9)
NT:P1.1	2	1			2	5 (6.0)
NT:P1.2			6		6	12 (14.5)
NT:P1.3					3	3 (3.6)
NT:P1.7					2	2 (2.4)
NT:P1.16	1					1 (1.2)
Total	25	7	13	2	36	83†

*No reaction with subtyping monoclonal antibodies. †One strain was not typed. NG Nongroupable; NT Nonserotypeable

Atlanta, Georgia). Mantel-Haenszel statistics were used to establish associations between the disease and risk factors. Relative risks and 95% confidence intervals were also calculated using the STATCALC program in EPIINFO. Fisher's exact test was used when expected cell frequencies in a 2x2 table were less than five. Two-tailed probabilities were used to establish significance.

RESULTS

Carriage rate: The carriage rates for all *Neisseria* species in the three areas surveyed ranged between 13.8% and 17.1% (Table 1). Southern Alberta had a statistically lower carriage rate for *N meningitidis* than both areas in Ontario.

In the three areas, age was the only factor consistently associated with colonization with *N meningitidis*. Age-adjusted carriage rates in persons younger than six years old were eight per 1000 and 241 per 1000 for *N meningitidis* and *Neisseria lactamica*, respectively. The rate of carriage for persons six years or older was 53 per 1000 for both *N meningitidis* and *N lactamica*. The majority of isolates of *N meningitidis* were obtained from individuals between 12 and 18 years of age in all three areas: Victoria County 30 of 35 or 86%; Perth County 24 of 34 or 71%; and Alberta 12 of 15 or 80%.

The following factors were not associated with an increased risk of colonization with either *N meningitidis* or *N lactamica*: symptoms of respiratory tract infections in the previous two weeks; underlying medical illness; number of persons living in the household; educational level of parents; and daycare attendance or participation in organized clubs or sports. In Victoria County, rifampin had been used by 23 of 493 respondents to the questionnaire. A significant association (two-tailed) was not found between rifampin use and carriage of any *Neisseria* species, but statistical power using these numbers was less than 10% and was, therefore, insufficient to detect a significant association if it exists.

Serogroups, serotypes and subtypes of *N meningitidis*: The distribution of *N meningitidis* by serogroup is shown in Table 2. Serogroup C and Y strains were isolated more frequently in Victoria County.

The distribution of sero- and subtypes among serogroups of *N meningitidis* is shown in Table 3. Overall, 17 combinations of serotypes and/or subtypes were found among the carrier strains. The most common serotypes, 4 and 15, were associated with 26.4% and 15.7%, respectively, of all strains. Nearly one-half of the strains were nonserotypeable. Serotype 4 was associated mainly with serogroup B and nongroupable strains, while serotype 15 was found most frequently among nongroupable strains.

The frequency of subtypes was as follows: P1.1 (9.6%), P1.2 (22.9%), P1.3 (3.6%), P1.7 (6.0%) and P1.16 (7.2%). Just under 50% of the strains failed to

react with the subtyping monoclonal antibodies. Fifteen of the 19 strains containing the P1.2 epitope were isolated in Victoria County. Fourteen of these strains were isolated from carriers aged 14 to 18 years; the 15th strain was isolated from a 26-year-old carrier. The P1.2 epitope occurred on three C:2a, one C:2b, one Y:2c, six Y:NT and four NG:NT strains isolated in Victoria County. Only two strains (Y:NT:P1.2) from Perth County and two strains (NG:NT:P1.2 and 29e:NT:P1.2) from southern Alberta contained the P1.2 epitope.

Electrophoretic analysis of enzymes: Enzyme electrophoretic analysis of the seven serogroup C carrier strains revealed that none was the same enzyme electrophoretic type as that causing the focal outbreak of disease in the Victoria County area. Enzyme electrophoretic patterns of representative strains of Y:NT:P1.2 and NG:NT:P1.2 isolated in the Victoria County area were identical to each other but were dissimilar from all serogroup C strains including that causing the outbreak.

DISCUSSION

The overall carriage rates for *N meningitidis* in this study were consistent with carriage rates of between 1% and 10% in other similar surveys (1,11,12). The difference in carriage rate between southern Alberta and Ontario was probably not due to differences in technique since comparable age-specific isolation rates for *N lactamica* were obtained. Carriage rates of the same serogroup of *N meningitidis* in household contacts and military recruits can be between 30% and 45% (11,13). The present study also reconfirmed that carriage of *N meningitidis* is more common in children over the age of six years, especially teenagers, while carriage of *N lactamica* is associated with younger age groups (14). Other factors associated with increased risk of carriage and/or disease have been crowded sleeping areas, upper respiratory tract symptoms, and coincident respiratory viral and mycoplasmal agents (13,15,16).

The unique aspects of this study are that it compared community carriage and clonality of group C meningococci in geographically separated outbreak and nonoutbreak areas. In England, outbreak strains of group B meningococci were found more commonly among carriers in housing estates with a higher incidence of disease, but other areas within the same city also had colonization with the same subtypes of organism (17). A Canadian carriage study was carried out in an isolated Arctic community with a high incidence of group B disease but comparisons between the disease-causing isolates and the carrier strains were not reported (18). Another random carrier study took place in the Port Hope and Cobourg areas of Ontario in September 1989. In that area the carriage rate for *N meningitidis* was 4% (54 of 1326). One individual had a group C isolate which was nontypeable and unrelated

to the outbreak strain according to enzyme electrophoretic analysis (personal communication).

The lack of carriage of the clone causing the outbreak in the Victoria County area is not unexpected. Very low carriage rates of outbreak strains of group B disease have been reported in England and Norway (19,20). Factors that could contribute to decreased carriage of outbreak strains in asymptomatic persons may include a shorter period of colonization or a rapid onset of clinical disease following acquisition of the organism in nonimmune hosts. Indeed, Edwards et al (21) have shown that carriage of group C strains causing invasive disease in military recruits is less than two weeks prior to development of clinical symptoms. Others have suggested that strains causing outbreaks or epidemics may be more virulent than strains responsible for sporadic disease (1,2). Proof of this would require longitudinal studies comparing morbidity and mortality associated with different strains of meningococci.

It appears that asymptomatic carriage may be associated with a diversity of serogroups and sero/subtypes of meningococcal strains in contrast to only a few sero/subtypes such as 2a:P1.2, 2b:P1.2, 2b:P1.Ham, 4:P1.15 and 15:P1.16, which have caused the majority of outbreaks of group B and C disease (7,10,19,22-24). In this study, none of the 12 combinations of serotype/nonserotype and/or subtypes identified among the 49 strains isolated in the two geographical areas free from disease was of the sero/subtypes mentioned above. Although not all carrier strains were examined using enzyme electrophoretic analysis, diversity in genetic relatedness has previously been shown to be greater in *N meningitidis* strains colonizing carriers compared to isolates from patients with meningococcal disease (25).

Acquired immunity to meningococcal disease depends in part on prior colonization with microorganisms in order to develop antibodies to group-specific polysaccharide and/or other cell surface components such as outer membrane proteins and lipopolysaccharides (lipo-oligosaccharides). Thus, an individual may be at greater risk of disease if not previously colonized with *N meningitidis* or *N lactamica* or other cross-reacting organisms (14,26). For *N lactamica*, the existence of common epitopes to meningococcal lipopolysaccharides may provide cross-immunity against meningococcal disease (27). In this study, 15 of 19 meningococcal strains (78.9%) containing the P1.2 epitope were isolated from carriers in the Victoria County. While it is unknown if these particular carriers had antibodies to the P1.2 epitope it is conceivable that they had developed immunity against group C organisms through carriage of nongroup C meningococci that were immunologically related through outer membrane proteins. Introduction of a strain of *N meningitidis*-carrying epitopes into a subgroup of a population not

previously exposed to a proportion of those antigens may partially explain the occurrence of localized outbreaks.

Techniques such as sero- and subtyping and enzyme electrophoretic typing have permitted the authors to establish that a homogeneous clone caused the outbreak in the Victoria County area and that the group C

carrier strains were infrequent, heterogenous and of differing subtypes and clones. By identifying clones of pathogenic meningococci that have caused outbreaks, it may be possible to look for virulence factors that characterize these organisms and to help clarify the complexities of meningococcal disease outbreaks.

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