Neisseria meningitidis Carriage during an Outbreak of Serogroup C Disease

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During 2001, an outbreak of serogroup C meningococcal disease led to immunization of individuals aged 13–29 years in Abbotsford, British Columbia, Canada. This study addresses the distribution of Neisseria meningitidis carriage in this population and the implications of that distribution for the targeting of the immunization campaign. Pharyngeal swabs were obtained at immunization from 2004 people. Colonies were identified and serogrouped using standard agglutination methods and by PCR. Isolates were characterized using pulsed-field gel electrophoresis (PFGE). The prevalence of N. meningitidis carriage was 153 carriers per 2004 subjects (7.6%; 95% confidence interval, 6.5%–8.9%). Only 6 (4%) of the isolates from these carriers were found to be serogroup C by agglutination or PCR testing, and all of these were from individuals within the age group targeted for immunization. Only 1 of these 6 isolates was found to be identical to the outbreak strain by PFGE. The observation that a virulent strain is not circulating widely suggests the possibility of low background immunity in the population at risk and emphasizes the importance of vaccination in controlling epidemic group C meningococcal disease.

From December 2000 through March 2001, 5 cases of invasive serogroup C meningococcal disease were identified in Abbotsford, British Columbia, Canada, with 1 resulting death (table 1). Abbotsford is an agricultural center (population, 121,689) located 71 km east of Vancouver in the Fraser Valley. In 4 of these 5 initial cases, the individual was in the 15–29-year age group (population, 24,899); the age-specific incidence during this period was 16 cases per 100,000 individuals. A fifth case in this age group resulted in a second fatality in early April 2001, during which time a public health response was being mobilized.

That public health response was an immunization strategy targeting those aged 13–29 years. The targeting was guided by the age distribution observed among the first 5 cases but also by the logistical and political expediency of including all, rather than a subset, of high school students in the campaign. Inclusion of younger students was felt to have the added benefit of providing protection to an age group to which the outbreak strain might be transmitted next. Teachers who were 1–29 years of age were not included, unless they worked in a school where a case had been reported. Because the provinces of Alberta and Quebec had reported continuing group C disease in some groups immunized with polysaccharide vaccine [1, 2], conjugated meningococcal C vaccine was used as soon as it was available for most of the British Columbia campaign.

Several questions emerged. Was the proposed campaign appropriately targeted? How widely distributed was carriage of the strain associated with invasive disease? Was a case of serogroup C meningococcal disease in a wood-mill worker from an adjacent region (who...
had negative results of culture for Neisseria meningitidis but in whom the disease was diagnosed by direct-specimen PCR) connected to the outbreak?

The primary objective of this study was to measure the prevalence of carriage of N. meningitidis in the group targeted for immunization, in groups aged <13 and ≥29 years, and among workers from the wood mill in a region bordering Abbotsford where one worker developed invasive serogroup C disease. Secondary objectives included identifying the demographic correlates of carriage and assessing the serogroup distribution of N. meningitidis isolates from carriers.

**METHODS**

Sampling. Recruiting occurred at special immunization clinics before each eligible person was vaccinated. Subjects included individuals who were eligible for meningococcal C vaccine (aged 13–29 years), those who were too old to be targeted (teachers at eligible high schools), and those who were too old to be targeted (teachers at eligible high schools). Recruiting was also directed at co-workers of the wood-mill employee who was ill at the same time that the outbreak occurred. After informed consent was obtained, pharyngeal swabs were collected, and information on age, sex, nonresidence in Abbotsford, and city of residence was recorded.

Microbiologic analysis. Specimens were transported to the laboratory in Amies charcoal transport medium and plated within 24 h after arrival on Thayer Martin medium. Colonies were identified using Gram staining, examination of colony morphology, and biochemical tests. Isolates identified as N. meningitidis were serogrouped at the British Columbia Centre for Disease Control (Vancouver) using an agglutination test. Antisera were obtained from Difco Laboratories for serogroups B and C and from the Health Canada National Microbiology Laboratory (Winnipeg, Manitoba) for serogroups A, 29e, W135, X, Y, and Z. Serogroup C isolates were forwarded to the National Microbiology Laboratory (Winnipeg, Manitoba) for serogroups A, B, C, W135, and Y. The DNA extract from each isolate was tested for amplification of the chaperonin 60 gene [5], to confirm that there were no false-negative PCR results from any of the individual DNA extracts.

**PFGE.** Isolates from invasive cases and throat isolates that were determined to be serogroup C by either the agglutination test or PCR were subjected to PFGE. In brief, we used a method described by the National Microbiology Laboratory, except that cells were treated with formalin. PFGE patterns were evaluated for relatedness using criteria established by Tenover et al. [6]. (A detailed description of the methods can be found in a technical appendix available from D.M.P.)

Statistical analysis. Simple proportions were calculated and 95% CIs were estimated using the exact binomial method. Assessment of risk factors was performed using MacNemar’s test (paired \( \chi^2 \) test) for dichotomous variables and a paired Student’s \( t \) test for continuous variables. Conditional logistic regression was used to determine what variables were independently associated with N. meningitidis carriage. Modeling involved the evaluation of first-order interactions.

**RESULTS**

Pharyngeal swabs were obtained from 2004 subjects (1847 of whom were within and 157 of whom were older or younger than the targeted age range). The overall prevalence of N. meningitidis carriage was 153 carriers per 2004 subjects (7.6%; 95% CI, 6.4%–8.8%). The distribution of carriage by age, sex, and city of residence in groups of subjects who were eligible and ineligible for immunization in the campaign is shown in table 2. The rate of carriage of N. meningitidis was significantly higher among male subjects and subjects in the vaccine-targeted age group. Unexpectedly, the rate of carriage was lower among residents of Abbotsford, where the group C meningococcal outbreak was concentrated, than among residents of nearby municipalities. Factors found by logistic regression to be independently associated with N. meningitidis carriage were male sex, nonresidence in Abbotsford, and age of 13–29 years (the age group targeted by the immunization program).

Serogroup distribution of isolates from pharyngeal swabs is shown in table 3. Cultures of samples from only 3 subjects were positive for N. meningitidis serogroup C. All 3 subjects were in...
Table 1. Characteristics of cases of invasive meningococcal disease in residents of Abbotsford, British Columbia, Canada.

<table>
<thead>
<tr>
<th>Case</th>
<th>Date of onset</th>
<th>Age, years</th>
<th>Sex</th>
<th>Residence</th>
<th>Neisseria meningitidis serogroup</th>
<th>N. meningitidis serosubgroup; ET</th>
<th>PFGE result</th>
<th>Gel lane</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12 Dec 2000</td>
<td>1</td>
<td>M</td>
<td>Abbotsford</td>
<td>C</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>24 Dec 2000</td>
<td>23</td>
<td>F</td>
<td>Abbotsford</td>
<td>C</td>
<td>C:2a,P1,15</td>
<td>Identical</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>12 Jan 2000</td>
<td>19</td>
<td>M</td>
<td>Abbotsford</td>
<td>C</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>12 Jan 2000</td>
<td>27</td>
<td>F</td>
<td>Abbotsford</td>
<td>C</td>
<td>C:2a,P1.5; ET15</td>
<td>Identical</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>23 Mar 2001</td>
<td>18</td>
<td>F</td>
<td>Abbotsford</td>
<td>C</td>
<td>C:2a,P1.5; ET15</td>
<td>Identical</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>5 Apr 2001</td>
<td>21</td>
<td>M</td>
<td>Abbotsford</td>
<td>C</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>12 Jun 2001</td>
<td>27</td>
<td>F</td>
<td>Abbotsford</td>
<td>C</td>
<td>C:2a,P1.5; ET15</td>
<td>Closely related</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>15 Jul 2001</td>
<td>22</td>
<td>F</td>
<td>Mission</td>
<td>C</td>
<td>C:2a,P1.5; ET15</td>
<td>Identical</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>30 Jul 2001</td>
<td>17</td>
<td>M</td>
<td>Abbotsford</td>
<td>C</td>
<td>C:2a,P1,2,5; ET15</td>
<td>Possibly related</td>
<td>6</td>
</tr>
</tbody>
</table>

NOTE. The cases that defined the original scope of the outbreak are in bold. ET, electrophoretic type; NA, not available.

* Compared with the outbreak strain.

** See Figure 1A.

† Diagnosis was confirmed by direct-specimen PCR only; no culture isolate was available for serosubtyping or electrophoretic typing.

‡ Fatal case.

§ Mission is a community adjacent to Abbotsford; the patient had work-related and social contact with individuals in Abbotsford.

Table 2. Prevalence of carriage of Neisseria meningitidis among participants in an immunization program in Canada.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of carriers/total no. of participants</th>
<th>Prevalence of carriage, % (95% CI)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>68/1134</td>
<td>6.0 (4.7–7.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>85/870</td>
<td>9.8 (7.9–11.9)</td>
<td>1.70 (1.20–2.40)</td>
<td>.002b</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11–12 years</td>
<td>1/82</td>
<td>1.2 (0.0–6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–29 years</td>
<td>147/1847</td>
<td>8.0 (6.8–9.3)</td>
<td>7.00 (1.20–282)</td>
<td>.001a</td>
</tr>
<tr>
<td>30–55 years</td>
<td>5/75</td>
<td>6.7 (2.7–14.5)</td>
<td>5.79 (0.62–277)</td>
<td>.023b</td>
</tr>
<tr>
<td>City of residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbotsford</td>
<td>99/1520</td>
<td>6.6 (5.4–7.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>54/484</td>
<td>11.2 (8.5–14.3)</td>
<td>1.82 (1.25–2.58)</td>
<td>.007</td>
</tr>
<tr>
<td>Immunization status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ineligible</td>
<td>6/189</td>
<td>3.2 (1.4–6.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eligible</td>
<td>147/1815</td>
<td>8.0 (6.9–9.4)</td>
<td>2.69 (1.18–7.55)</td>
<td>.007a</td>
</tr>
</tbody>
</table>

* Calculated using the likelihood ratio test.

** Calculated using the χ² test.

† Those ineligible for immunization included individuals who were older or younger than the target age groups and 32 wood-mill workers who were within the target age group but were residents of adjacent communities.

The 13–29-year-old immunization target group. An additional 3 nongroupable isolates were non–capsule expressing but were found by PCR to have biosynthetic genes for serogroup C. These isolates were from subjects in the same age group, although 2 of the 3 subjects were from communities adjacent to Abbotsford.

Figure 1A shows the PFGE patterns of 6 serogroup C isolates obtained from subjects who developed invasive meningococcal disease as part of this outbreak. Isolates in lanes 1–4 are identical. Based on the Tenover criteria, the isolate in lane 5 is closely related to the isolates in lanes 1–4 (3 bands are different), and the isolate in lane 6 may be related to the isolates in lanes 1–4 (4 bands are different).

Figure 1B shows the PFGE patterns of 6 serogroup C isolates identified in the carrier prevalence study. Isolates in 3 of these lanes (lanes 1, 3, and 5) were identified by the agglutination
A similar inability to find carriage of the epidemic strain in high– and low–disease incidence areas [9]. A similar inability to find carriage of the epidemic strain in high– and low–disease incidence areas [9].

There are several possible explanations for such findings. First, because the study was launched after the outbreak had been established and as vaccine was being distributed, maximum community carriage rates may have preceded the study. Second, some genetic serogroup C isolates are capable of switching their capsular expression on and off [11]. By this mechanism, it is possible that some serogroup C strains could not be identified by phenotypic methods. Our use of PCR to aid serogrouping increased the number of serogroup C isolates identified, but identification of such isolates still was very rare. Finally, nasopharyngeal swab specimens have proven to be insensitive, and it is unlikely that pharyngeal swabs would perform better [12].

It is likely that low rates of carriage result in low rates of protective immunity against virulent strains, contributing to susceptibility of the population to outbreaks. It has been reported that low prevalence of carriage of an N. meningitidis outbreak strain, together with a low prevalence of protective immunity within a student population, was associated with a high incidence of invasive disease among those who acquired the strain [7]. Although immunity was not assessed in the present study, the consistent finding of a low rate of carriage of serogroup C in the presence of heightened disease activity suggests that immunization has a particularly important role in preventing morbidity resulting from serogroup C disease.

The findings of this study did not point to a need to retarget immunization programs in Abbotsford. Epidemic strain serogroup C carriage was only detected in targeted age groups, although it was rare enough that carriage might have been present but unidentified in other groups. However, our findings did prove useful in alerting public health personnel to a connection between an apparently sporadic case in a wood-mill worker from an adjacent region and the Abbotsford outbreak. Even though this patient had a clinical diagnosis of meningococcal disease confirmed by direct-specimen PCR, identification of carriage of the outbreak strain in a close coworker supported a connection to the Abbotsford outbreak. Had further cases developed in that context or community, the immunization program would have been more rapidly implemented as a result of our findings.

This study has several limitations. First, it was cross-sectional, and, therefore, transient carriage would not have been detected. Some isolates that were not groupable by phenotypic methods might have been found to be serogroup C by genetic methods, but this problem has been minimized by use of PCR typing. We did not assess immunity to serogroup C by serological means, and thus we were unable to correlate immune status with pharyngeal colonization. This outbreak also may not have been as widespread as some outbreaks associated with serogroup C. The US Centers for Disease Control and Prevention recommend interventions when the rate exceeds 10 cases per 100,000 individuals within 3 months [13]. This is often interpreted by crude analyses without stratification by age (which was used in our analysis of data from Abbotsford). However, we also assessed whether a significant age-stratified increase was present using the Poisson distribution and found that the third case to occur within the 3-month limit was a significant aberration (P<.05).

In this study, detection of an outbreak-associated serogroup C strain in asymptomatic carriers was rare. This observation, together with the association between low rates of carriage and immunity and the risk of invasive serogroup C disease described

### DISCUSSION

N. meningitidis serogroup C carriage was found rarely during an intervention for an established outbreak. Only 0.15% of subjects carried group C isolates that were detectable by culture and agglutination testing; that rate increased to 0.3% when PCR was used. This finding is consistent with previous studies of N. meningitidis carriage during outbreaks, in which the prevalence of carriage ranged from 0.5% to 1.7% [7, 8]. In one study, carriage of the epidemic strain was not found in the most affected age group, and there was no difference in the carriage rate of the epidemic strain in high– and low–disease incidence areas [9]. A similar inability to find carriage of N. meningitidis group C has been reported in a hypersporadic county in Georgia [10].

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Meningococcal Carriage during an Outbreak

Figure 1. A, PFGE patterns of 6 Neisseria meningitidis serogroup C isolates obtained from individuals with invasive meningococcal disease involved in an outbreak in Abbotsford, British Columbia, Canada, in 2001. The isolates in lanes 1–4 are identical, the isolate in lane 5 is closely related to those 4 isolates (3 bands are different), and the isolate in lane 6 may be related to the isolates in lanes 1–4 (4 bands are different). B, PFGE patterns of 6 N. meningitidis serogroup C isolates identified in a carrier prevalence study. Three of these (lanes 1, 3, and 5) were identified by the agglutination test; the remainder (lanes 2, 4, and 6) were identified by PCR. Only an isolate from a wood-mill employee from an adjacent region (lane 1) was identical to the outbreak strain (A, lanes 1–4).

Acknowledgments

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References

2. Tyrrell GJ, Chui L, Johnson M, Chang N, Rennie RP, Talbot JA. Out-

elsewhere [7], underscores the importance of targeted immunization in containing serogroup C meningococcal disease once an epidemic threshold has been crossed.


