Molecular Epidemiology of Sporadic (Endemic) Serogroup C Meningococcal Disease

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Understanding the basis of sporadic (endemic) meningococcal disease may be critical to prevention of meningococcal epidemic outbreaks and to understanding fluctuations in incidence. Active, prospective, population-based surveillance and molecular epidemiologic techniques were used to study sporadic serogroup C meningococcal disease in a population of 2.34 million persons (Atlanta area). During 1988–1994, in which no outbreaks or case clusters were reported, 71 patients developed sporadic serogroup C meningococcal disease (annual incidence, 0.51/100,000). Eighty-three percent of patients were >2 years old. By multilocus enzyme electrophoresis, pulsed-field gel electrophoresis, and serotyping, 84% (52/62) of the isolates available for study were identical or closely related members of the electrophoretic type 37 (ET 37) complex responsible for multiple serogroup C outbreaks in the United States in the 1990s. Sporadic disease caused by 9 clonal strains occurred over periods up to 4 years and accounted for 45% (28/62) of cases. Sporadic serogroup C meningococcal disease was most often due to a limited number of related strains that appear to slowly circulate in the population.

Infections due to Neisseria meningitidis remain a serious health problem. Over 350,000 cases occur worldwide each year. Currently, the majority of meningococcal infections in the United States and other developed countries are due to capsular serogroups B, C, Y, and W-135 [1], but the age-specific and serogroup-specific incidence of meningococcal disease due to these serogroups is subject to considerable fluctuations [1–3]. Since 1990, there has been a dramatic increase in the number of local outbreaks caused by capsular serogroup C meningococci in the United States [4] and an increased incidence of serogroup C disease has occurred in Canada [5, 6]. In addition, disease caused by serogroup C meningococci related to outbreak-associated serogroup B strains (electrophoretic type [ET] 5 complex) has been noted recently in the Pacific Northwest [7, 8]. However, >90% of all cases of serogroup C meningococcal disease in the United States occur as sporadic (endemic) disease [1]. The characteristics of the serogroup C strains causing sporadic meningococcal disease and the relationship between epidemic outbreaks and sporadic disease in a population are not established. Are strains causing sporadic meningococcal disease and epidemic outbreaks related or different? Also unexplained are the episodic fluctuations in the age-specific incidence of serogroup C meningococcal disease [1–3].

The primary aim of this study was to understand better the role of specific serogroup C meningococcal strains in sporadic and epidemic disease. We used data from active, prospective, population-based surveillance; hospital patient chart review; and typing of isolates by multilocus enzyme electrophoresis (MEE), pulsed-field gel electrophoresis (PFGE), and serology. Secondary aims were to compare PFGE with other typing methods for N. meningitidis, to identify differences in clinical manifestations among patients with serogroup C N. meningitidis, and to study transmission of serogroup C meningococcal strains in a defined population.

Methods

Collection of cases and demographic data. Patients with invasive meningococcal disease who were residents of the Atlanta metropolitan area (Georgia Health District III) were identified by active surveillance at all 35 hospitals and a major referral laboratory serving the population of 2.34 million from December 1988 to December 1994. Invasive meningococcal disease was defined as patients with a compatible clinical syndrome and laboratory evidence of N. meningitidis at a sterile site. (In >98% of cases, this was a growth of N. meningitidis in a culture of blood or cerebrospinal fluid.) Surveillance was performed prospectively as part of an active bacteremia and meningitis surveillance project carried out in conjunction with the Georgia Department of Human Resources and the Centers for Disease Control and Prevention (CDC) [9–12]. Initial case reports of invasive meningococcal disease were obtained biweekly from two independent sources: the hospital microbiology laboratories that provided the isolates and the hospital infection control practitioners. Laboratory audits at
all hospitals every 6 months evaluated accuracy of reporting and identified any cases that were not reported initially.

Sterile-site (e.g., blood or cerebrospinal fluid) isolates of *N. meningitidis* were initially identified at local hospital laboratories by standard methods. A case report form including demographic information was available for each identified case. Population data from a 1990 census were used to calculate overall disease incidence and incidence by specific age-groups or postal zip codes. No epidemic outbreaks or case clusters of meningococcal disease were reported in the surveillance population during the study period. However, the western boundary of the surveillance population was 48 km from an area (Carrollton, GA) that experienced an outbreak of serogroup C meningococcal disease in 1992–1993.

**Collection of isolates, serogrouping, and serotyping.** Isolates were initially confirmed as *N. meningitidis* by standard methods (e.g., gram-negative diplococci, positive oxidase test, utilization of glucose and maltose). Isolates were also subcultured and in some cases serogrouped at each hospital laboratory. In >80% of cases, a viable isolate was subsequently collected from these laboratories and was stored at −70°C in gonococcal broth with 16% glycerol.

Serogroup was confirmed and serotype and serosubtype were determined for these isolates as follows: Serogrouping was performed by standard slide agglutination. All strains were serotyped with monoclonal antibodies (MAbs) for outer-membrane protein (OMP) class 2/3 epitopes 2.2a, 2.2b, 3.4, and 3.15; subtyped for OMP class 1 epitopes P1.2, P1.5, P1.7, P1.16; and immunotyped for lipooligosaccharide (LOS) epitopes L8 and L3.7.9. If negative for these MAbs, strains were further tested with MAbs for serotypes 2.2c, 3.1, 3.11, 3.14, and 3.21; serosubtypes P1.1, P1.3, P1.4, P1.6, P1.9, P1.10, P1.12, P1.13, and P1.15; and LOS types L1 and L10. MAbs were obtained from the National Institute for Biological Standards and Control (Potters Bar, UK). The serotyping procedure [13] was modified to grow meningococci on brain-heart infusion medium (Difco, Detroit) with 1% horse serum (GIBCO Laboratories, Grand Island, NY) and to use a higher concentration of cells (cell density, 1.0 at *A*soo), a different blocking buffer (PBS + 0.1% Tween 20), and a shorter primary antibody incubation (2.5 h).

**MEE methods.** MEE was carried out by established methods [14, 15]. Briefly, constitutive cytosolic enzymes were extracted and run in 11% starch gels. The band position for each of 24 cellular enzymes was established by specific staining for each enzyme. Relative enzyme mobilities (alleles) were numbered in order of increasing anodal migration, and each unique set of alleles was defined as an ET.

**PFGE methods.** PFGE was carried out as previously described [16]. After each isolate was streaked for purity, overnight cultures on gonococcal agar were suspended in TRIS buffer, then resuspended in 1% low-melt agarose, and solidified into plugs. Chilled agarose plugs were then successively suspended in lysozyme solution, proteinase K solution, and five wash steps in TRIS-EDTA buffer. After overnight digestion with restriction enzyme, plugs were electrophoresed in a pulsed-field apparatus (CHEF 2; Bio-Rad Laboratories, Richmond, CA) by using a ramped field for 20 h. Gels were photographed under UV illumination. Strain GA1655, the first ET 24 strain isolated in the surveillance, and a λ-phage fragment molecular-weight ladder (Lambda Ladder PFGE Marker; New England BioLabs, Beverly, MA) were run on each gel to standardize comparison of bands on different gels. Initial experiments showed three enzymes (*Nhe*I, *Sfi*I, *Bgl*III) to be most discriminative; electrophoresis of all available serogroup C isolates was subsequently carried out with *Nhe*I and *Sfi*I. For each meningococcal isolate, the list of bands present (1) or absent (0) at each band position in the gel was determined; each unique set of bands was defined as a PFGE type.

**Clinical definitions.** We reviewed the medical records of all patients with serogroup C meningococcal disease to determine clinical features and underlying conditions. Case mortality was obtained from hospital charts and surveillance. The determination of clinical syndrome (e.g., meningococcemia and meningococcal meningitis) was made by using established definitions [9].

**Data analysis.** Clinical and laboratory data were collated and analyzed by using Epi Info software (version 6.02; CDC). The number of observed cases for each zip code was compared with the number of cases expected (based on the total number of cases and population for the whole surveillance area) using the Poisson distribution.

Dendrograms of the ET and PFGE patterns were produced by the unweighted pair group method for arithmetic averages [17, 18]. Analysis was based on whether isolates were similar or different at each enzyme (ET) or band (PFGE) position. Less common differences were weighted to indicate a greater genetic distance [15].

**Results**

**Demographics.** During the surveillance period, December 1988 through November 1994, 151 patients with invasive meningococcal disease were identified in the population; serogroup data were reported for 135 of these. Serogroup C *N. meningitidis* caused disease in 71 patients (70 were identified by growth of *N. meningitidis* in a sterile-site culture, and 1 patient with a clinical syndrome of meningitis was identified by Gram’s stain of cerebrospinal fluid showing gram-negative diplococci and a positive cerebrospinal antigen for serogroup C meningococcal capsular polysaccharide). The average annual incidence of serogroup C disease was 0.51/100,000. Serogroup B *N. meningitidis* caused disease in 40 patients; 18 were serogroup Y and 6 were serogroup W-135. In 62 (87%) of the 71 patients with serogroup C, an isolate was available for confirmation of the serogroup and further typing. In contrast to serogroup B disease, which caused disease predominantly in children <2 years old, 72% (51/71) of serogroup C disease occurred in children and young adults 2–29 years old (odds ratio, 3.3; 95% confidence interval, 1.3–8.5). Overall, 83% of serogroup C disease occurred in those >2 years old. Nine deaths occurred in the serogroup C–infected patients, for an overall mortality for serogroup C of 12.7%. Peak serogroup C disease incidence was in March (12 cases); 76% of cases occurred from January to July. There was no significant difference in the incidence of serogroup C according to sex or race.

Six adjacent zip code areas in the western part of the surveillance area each had significantly more serogroup C cases than predicted by their populations (*P* < .05, Poisson distribution, Epi Info, v. 6.2). Combined, this region had 21 (30%) of the
71 cases of serogroup C disease, but no more than 5 cases occurred in any 1 year. The observed (σ) incidence (2.1/100,000) was 4.1 times higher than expected (E) for the population of 195,000. Further, the incidence of meningococcal disease caused by serogroups B and W-135 was also increased 21, and 22, and ET 27 (mean, 9) were noted using NheI and SfiI were used. Some isolates of ETs 13, 17, and 27 had PFGE types that differed by ≥4 bands (e.g., ET 13 [NheI PFGE types 13, 19, and 22], ET 17 [NheI PFGE types 5, 6, 11, 12, 14, 20, 21, and 22], and ET 27 [NheI PFGE types 7, 9, 10, 17, and 18]). Conversely, some isolates with different ET types could not be distinguished by PFGE, for example, NheI PFGE type 19 (ETs 13 and 24) and NheI PFGE type 22 (ETs 13 and 17). Of 52 isolates studied by PFGE that belonged to the cluster of related ET 37 complex types (ETs 13, 17, 24, 27, 33, 146) described above, 51 differed by a genetic distance by PFGE of <0.41 in the NheI dendrogram (figure 2B, bold) and <0.64 in the SfiI dendrogram (data not shown).

Serotyping. Of the 62 strains, 39 were serotype 2a, 13 were serotype 5, and 6 strains were type 2b (n = 1), type 4 (n = 3), type 14 (n = 1), or type 15 (n = 1); 4 were nontypeable. Fifty-nine strains were subtypeable. Fifty-three of these reacted to either P1.2 or P1.5, and 46 strains reacted to both. Remaining subtypes identified were P1.6 (n = 1), P1.7 (n = 4), P1.12 (n = 1), P1.13 (n = 1), P1.15 (n = 2), and P1.16 (n = 1). Fifty-four strains expressed L3,7,9 LOS reactivity, while 16 reacted to both L8 and L3,7,9 MAbs. High-frequency phase variation is known to occur between the L8 and L3,7,9 LOS structures [21].

Of 53 strains, 43 (81%) belonging to the ET 37 complex types 13, 17, 24, 27, 33, and 146 were serotype:subtype 2a:P1.2,5 (figure 3) or 5:P1.2,5. The remaining 10 strains of these ET types displayed either the 2a serotype or the P1.2,5 subtype epitopes. None of the strains outside the ET 37 complex group expressed the 2a:P1.2,5 or 5:P1.2,5 serotype:subtype. These strains displayed variable serotypes (e.g., 14, 4, 15) and subtypes (e.g., P1.15, 1.6, 1.7, 1.12, 1.16). LOS immunotype (L3,7,9 or L8, L3,7,9) was similar for both ET 37 and non-ET 37 strains.

Comparison of typing systems for sporadic group C meningococcal strains. The genetic-based typing systems (ET and PFGE) and serotyping indicated that the majority of cases of sporadic serogroup C meningococcal disease in metropolitan Atlanta were due to identical or closely related strains. Nine clonal strains — defined as identical by ET type, PFGE type, serotype, subtype, and immunotype (figure 3, bold type and clone numbers) — accounted ≥2 sporadic cases and accounted for an estimated 45% (28/62) of serogroup C disease in metropolitan Atlanta. With genetic relatedness defined as a genetic distance of ≤0.075 by ET (figure 1), a genetic distance of <0.41 by PFGE with NheI (figure 2B) or <0.64 with SfiI, and identical or related serotype:subtype serology, isolates were further divided into 9 groups (I–IX, figure 3). Group I, all of which were ET 37 complex strains, contained the 28 identical isolates of the 9 clones noted above and 24 related isolates (figure 3). Group I strains caused an estimated 84% (52/62) of sporadic serogroup C meningococcal disease in Atlanta. In contrast, groups II–IX, many of which contained only 1 isolate, were distinct from group I strains and from each other (figure 3).
Comparison of clinical manifestations. When demographic and clinical features of disease caused by group I strains were compared with those caused by group II–IX strains, there was no difference by sex or seasonal pattern (table 1). However, group I strains were more likely to cause disease in whites than in blacks ($P = .05$ [2-tailed Fisher’s exact test]). A trend toward more disease in those >30 years old and more severe manifestations (e.g., intensive care unit [ICU] admission) with group I strains was also observed. Mortality was 15% for the group I strains (8/52 died) compared with 0% for the group II–IX strains (0/10 died). Defined risk factors (e.g., complement or immunoglobulin deficiency) were not noted in any group. Underlying illness or conditions were similar for group I and groups II–IX.

Temporal and geographic relationships of sporadic group C strains. Serogroup C meningococcal strains of group I—identical by MEE, PFGE, and serotyping—caused cases of disease that occurred within days or weeks of each other (e.g., isolates 1791 and 1794; 1298 and 1301; 504 and 519) but also caused sporadic disease in Atlanta over periods of several months to as long as 4 years (figure 3). For example, 4 unlinked, sporadic cases (clone 2; isolates 549, 1843, 2487, and 3165) were caused by ET 17, serotype 2a:P1.2,5:L3,7,9; NheI and SphI PFGE identical isolates occurred between July 1990 and April 1994 (figure 3).

For most strains, there was no identifiable geographic pattern of spread. However, for 1 strain spread from a nearby epidemic, focus outside the surveillance area was noted. An outbreak of serogroup C, ET 24 meningococcal disease in a college community in Carroll County, west of Atlanta, involved 11 cases from December 1992 to April 1993. Four cases of meningococcal disease caused by ET 24 isolates but no case clusters or outbreaks occurred in the Atlanta surveillance area from December 1992 to February 1994. The first of these cases (isolate 1655) was a Carrollton college student who lived in Atlanta. The other 3 cases occurred throughout the surveillance area; the patients had no known interaction with the Carrollton epidemic area and no known association with each other.

When analyzed by MEE, PFGE, and serotyping, the 21 serogroup C isolates from the western part of the surveillance area with
Figure 3. Serogroup C Neisseria meningitidis causing sporadic cases of meningococcal disease in Atlanta, 1989–1994. PFGE, pulsed-field gel electrophoresis; ET, electrophoretic type. * See text.

increased incidence of cases were diverse (6 ETs, 11 types by NheI and 9 by SfiI). However, multiple (up to 3) isolates were identical by the combined typing methods (clones 1, 3, 5, 6, and 8, figure 3), accounting for 12 of the 21 cases. The cases caused by identical isolates in this area were separated by as long as 22 months. None of these cases were contacts or linked to another case.

Discussion

During our 6-year active population-based surveillance, in which no epidemic outbreaks or case clusters were reported, 71 patients with sporadic serogroup C meningococcal disease were identified in metropolitan Atlanta, for an average annual
incidence of 0.51/100,000 and a mortality rate of 12.7%. These rates are similar to those recently noted elsewhere in the United States [1, 3] for sporadic serogroup C meningococcal disease. Also consistent with nationwide trends during the surveillance [1, 3], the age distribution for serogroup C disease differed from that of serogroup B. The majority of serogroup C disease was seen in children >2 years old, teenagers, and young adults. Increased disease rates in older children and adolescents occur preceding or during meningococcal epidemics [22] and have also been associated with cyclic increases in serogroup-specific meningococcal disease [2, 7]. Although the overall incidence of serogroup C disease did not increase during the 6-year surveillance and has not increased in the surveillance area in the year since the study was completed (unpublished data), outbreaks of serogroup C disease have occurred in many parts of the country in the 1990s [4]. The age distribution of serogroup C meningococcal disease in older children and adults, the hyperendemic rates of meningococcal disease in one area of the surveillance population, and the increased number of outbreaks in the United States are characteristics of meningococcal disease caused by a limited number of strains of high invasiveness [22].

By three different typing systems, 85% of serogroup C isolates causing sporadic disease in Atlanta were identical or closely related. Epidemiologic typing of *N. meningitidis* has traditionally been carried out by serologic typing (e.g., serogroup, serotype, serosubtype, immunotype). More recently, genetic methods such as ET [14, 15] and PFGE typing have been applied [6, 16, 23, 24]. Serogrouping of capsule or serotyping of major OMPs and LOS can distinguish meningococcal strains, but the results may be affected by rapid phase or structural variation in these surface components. This is due to high-frequency genetic events (e.g., slip-strand mispairing, rearrangements, and horizontal genetic exchange caused by transformation) common to *Neisseria* species [8, 25–27]. Thus, serology should be used with caution as a marker for individual clones and in detecting genetic relationships between meningococcal strains causing sporadic disease. Most of the related strains were serotype 2a and subtype 1.2,5, characteristic of a group of related strains designated the ET 37 complex [19].

In MEE typing, variations in the electrophoretic mobility of individual constitutive enzymes reflect substitution of amino acids of a different charge, which is a phenotypic measure of major genetic variations in bacterial “housekeeping” genes not under selective pressure. Electrophoretic typing has provided quantitative data regarding the population genetics of meningococci and other bacterial pathogens [28, 29] and has been shown to be discriminative in epidemiologic studies [23]. As suggested by the serotype and subtype data, most of the serogroup C meningococcal disease in Atlanta was caused by strains comprising 5 closely related (figure 1) ETs of the ET 37 complex. Strains of these same ETs were the cause of 8 of the 12 outbreaks of serogroup C meningococcal disease in the United States between 1990 and 1993 [4]. In addition, the predominant serogroup C strain responsible for the increased incidence of meningococcal disease in Canada (ET 15 in the Canadian system) [6] is an ET 37 complex strain that has the ET 24 profile designation in the United States.

PFGE has been recently used as an alternative genetic typing technique in analysis of meningococci [6, 16, 24] and has identified closely related meningococcal strains, such as those isolated from a single outbreak or those with a defined epidemiologic association. After digestion of the whole bacterial chromosome with a restriction enzyme with infrequent cleavage sites, up to 30 bands are distinguishable on electrophoresis. Both PFGE and MEE methods have the advantage that all strains are typeable, but PFGE is technically simpler. Restriction enzymes previously identified as potentially useful in the PFGE analysis of *N. meningitidis* strains have included *Sfi*, *Nhe*, *Spe*, *Not*, *Pac*, and *Bgl* [6, 16, 24]. Important to interpretation of data generated by both PFGE and MEE from different laboratories is standardization of the housekeeping enzymes used for MEE and the restriction enzymes used for PFGE.

In the present study of sporadic meningococcal disease, we found that PFGE typing with *Nhe* best identified major groupings distinguished by ET and subgroups within ETs. For example, strains of the ET 37 complex comprised 23 PFGE types. Thus, PFGE may be more discriminatory than MEE. However, large differences in PFGE patterns may be caused by relatively small changes in the chromosome [16]. If major PFGE groups are defined by differences of ≤6 bands (*Nhe* average genetic distance of ≤0.28, figure 2B), criteria that define related strains in outbreak settings [20], then 6 PFGE groups represent the 5 common ET 37 complex ETs identified in our study.

When cases of serogroup C meningococcal disease caused by the closely related (ET 37 complex, group I) strains were compared with cases caused by the genetically diverse isolates of groups II–IX, the group I strains caused more disease in whites versus blacks. In addition, there was a trend toward a higher proportion of cases caused by group I strains presenting with ICU admission and resulting in a fatal outcome. In Canada, multiple small outbreaks and the overall increased incidence of serogroup C disease have been attributed to the spread of virulent ET 37 clonal complex isolates, predominantly C:2a, P1.2,13, ET 15 (24) [5, 6]. Disease with these strains has been associated with a higher mortality [5].

Our finding of a limited number of identical or closely related meningococcal strains causing most cases of sporadic meningococcal serogroup C disease and over one-third of all sporadic cases of meningococcal disease in Atlanta between 1989 and 1994 should be considered in the context of meningococcal carriage. The overall nasopharyngeal carriage of *N. meningitidis* in a population during periods when the organism is endemic is ~10%. However, when compared with the nasopharyngeal carriage of serogroup B (3.1%), serogroup Y (1.9%), and nongroupable strains (4.8%), serogroup C meningococci are infrequently (0.3%–0.5%) isolated from the human naso-
Pharynx [30–34]. In a large, population-based study of nasopharyngeal carriage in Norway that utilized ET typing [31], only 4 serogroup C carrier strains were isolated, 2 of which belonged to the ET 37 complex. In a study of >5000 persons in the United Kingdom [30], nasopharyngeal carriage of serogroup C was 0.37%, and only 5 of the 19 serogroup C isolates recovered were serotypeable. Similarly, at the University of Illinois, among 867 asymptomatic college students, the overall meningococcal carriage was 9.9%; 0.3% of these were serogroup C carriers and 1 (0.17%) carried an ET 37 outbreak-associated strain [33]. If we extrapolate a 0.4% annual serogroup C meningococcal carriage, of which strains half or less are ET 37 complex strains, to our population of 2.34 million, then the yearly ratio of serogroup C carriers to cases of invasive serogroup C disease is 40:1 for the ET 37 complex strains and 2300:1 for the other serogroup C strains. In contrast, the ratios of carriage to invasion for serogroup B and Y strains are 9100:1 and 12,400, respectively. Thus, it appears that sporadic meningococcal disease occurs with increased frequency following transmission of serogroup C N. meningitidis and especially serogroup C meningococcal strains of the ET 37 complex.

The relatedness of the meningococcal isolates causing endemic serogroup C disease in our defined population differs from the diversity observed among known serogroup C strains from diverse geographic origins. Caugant et al. [28] studied 108 randomly collected, mostly disease-causing serogroup C strains from worldwide sources and noted 57 distinct ETs with a large genetic diversity. However, they noted that genotypes identified in one geographic region at a given time tend to be closely related and may persist over long periods of time. These data and ours are consistent with the concept of gradual evolution (years) and slow spread of virulent serogroup C meningococcal clones in geographically defined human populations.

Serogroup C meningococcal strains that were identical by MEE, PFGE, and serotyping caused sporadic meningococcal disease over weeks, months, and periods as long as 4 years in the Atlanta area. This also suggests prolonged asymptomatic carriage of disease-causing strains or slow transmission in “normal” populations (or both). In contrast to high rates of meningococcal carriage and transmission in closed populations, such as military recruits, our observations are consistent with studies in nonepidemic settings by Rake [35], who noted meningococcal carriage of up to 2 years in a single individual [35], and Greenfield et al. [32], who noted that the median duration of meningococcal carriage is 9.6 months and that meningococcal transmission even within families is infrequent. In support of this model, we noted the introduction into the surveillance population of a serogroup C, ET 24 (ET 37 complex) clone which caused a meningococcal outbreak in a nearby college community but which did not cause epidemic outbreaks or case clusters in the Atlanta population over the next 2 years. The self-limiting nature of many ET 37, serogroup C outbreaks has been previously noted [4], but the reasons for their appearance or disappearance are not known. We suggest that serogroup C outbreaks result when transmission or invasiveness of slowly circulating virulent meningococcal clones is enhanced by the presence of cofactors that have been epidemiologically associated with disease, such as crowding, smoking and coinfections.

The significant increase in serogroup C meningococcal disease outbreaks in North America was mostly caused by related strains [4], which previously constituted only a very small proportion of disease—presumably strains that were “newly” entering the population [5]. The epidemics were associated with classical age shift to older children, again theoretically explained by introduction of new strains into susceptible populations. At the same time, endemic or sporadic disease proceeded in most areas at usual rates. Our study showed that most of this endemic disease was also caused by the same strains that caused the outbreaks. There were no outbreaks in the study area, but disease caused by epidemic clones was associated with an age shift to older children and adolescents, suggesting that the new strains were causing disease in a wider swath of the population (consistent with introduction of new strains into susceptible populations) but not outbreaks. Presumably, this is because other epidemiologic criteria for outbreaks (e.g., smoking, crowding) were not met. As the clone remains in the population for a prolonged period, it is predicted that the age distribution will shift toward younger ages until the next new clone arrives.

In summary, data concerning the genetic relationships of serogroup C N. meningitidis have come largely from the study of isolates from epidemic outbreaks or case clusters. These isolates often belong to clonal groupings, but the overall population of serogroup C meningococci is thought to be nonclonal [29]. Our data indicate that the meningococcal strains causing sporadic meningococcal serogroup C disease in Atlanta over a 6-year period were limited in number and usually highly related. These virulent strains slowly circulated in the population and caused the majority of sporadic disease. When transmission of these strains increases or the acquiring population has increased susceptibility, period epidemic outbreaks or case clusters can occur.

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References