Outbreaks of invasive pneumococcal disease (IPD) caused by *Streptococcus pneumoniae* serotype 12F were observed in two neighboring regions of rural Alaska in 2003 to 2006 and 2006 to 2008. IPD surveillance data from 1986 to 2009 and carriage survey data from 1998 to 2004 and 2008 to 2009 were reviewed to identify patterns of serotype 12F transmission. Pulsed-field gel electrophoresis was performed on all available isolates, and selected isolates were characterized by additional genetic subtyping methods. Serotype 12F IPD occurred in two waves in Alaska between 1986 and 2008. While cases of disease occurred nearly every year in Anchorage, in rural regions, 12F IPD occurred with rates 10- to 20-fold higher than those in Anchorage, often with many years between disease peaks and generally caused by a single predominant genetic clone. Carriage occurred predominantly in adults, except early in the rural outbreaks, when most carriage was in persons <18 years old. In rural regions, carriage of 12F disappeared completely after outbreaks. Different 12F clones appear to have been introduced episodically into rural populations, spread widely in young, immunologically naïve populations (leading to outbreaks of IPD lasting 1 to 3 years), and then disappeared rapidly from the population. Larger population centers might have been the reservoir for these clones. This epidemiologic pattern is consistent with a highly virulent, but immunogenic, form of pneumococcus.

*S. pneumoniae* is a major cause of bacterial pneumonia and meningitis in persons of all ages and is the target of universal immunization programs for children and elderly adults in the United States (1, 2). In spite of high immunization rates with conjugate vaccine in American Indian and Alaskan native (AI/AN) children 19 to 36 months of age (92.6% received 3 doses) (3) and with pneumococcal polysaccharide vaccine among AI/AN adults (>90% received at least one dose) (4), rates of invasive pneumococcal disease (IPD) in children in this age group in the AI/AN population in Alaska are markedly higher than those in the non-Hispanic white Alaska population (64.6% received at least one dose of vaccine) and in the overall U.S. population (70.5% received at least one dose of vaccine) (3). Previous studies identified a number of risk factors for IPD in AI/AN populations, including increased household crowding, day care attendance, and lack of in-home piped water (5, 6).

While most cases of IPD are sporadic in nature and outbreaks of IPD (most of which have involved small, closed populations) have not played a major role in the recent epidemiology of pneumococcal disease (7), community outbreaks of disease have been reported from rural Australia (8, 9) and Northern Canada (10). Between 2003 and 2006, we observed a community-wide outbreak of IPD caused by *Streptococcus pneumoniae* serotype 12F with a previously unidentified unique pulsed-field gel electrophoresis (PFGE) pattern in one rural Alaska area (Fig. 1, region A) (11). Between October 2006 and July 2008, 21 cases of 12F IPD occurred in a neighboring region (Fig. 1, region B) where no cases of 12F IPD had been identified in the previous 15 years. Serotype 12F has been associated with outbreaks of disease (12), and we were concerned about the possibility that the unique clone of 12F isolated in the region A outbreak would spread to new regions. We reviewed statewide IPD surveillance data, case report information, carriage data, and genetic subtyping results to characterize these 12F IPD isolates and the epidemiology of 12F disease in Alaska.

**MATERIALS AND METHODS**

**Surveillance.** The 2010 U.S. Census reports a total population of 710,231 residents in Alaska, 65% (459,449) of whom live in the largest metropolitan area (Anchorage) and surrounding communities (13). The remaining population lives in smaller towns or villages. Most of these largely rural regions (Fig. 1, regions A through G) have populations of fewer than 30,000 people, but two regions (G and F) have larger populations of 106,436 and 67,884 people, respectively. No roads connect the rural western regions (regions A, B, C, or E) with the rest of the state; travel to and from these areas is primarily by air, with AI/AN persons comprising the majority of the population.

Since 1986, cases of IPD (defined as isolation of *S. pneumoniae* from a normally sterile site in an Alaska resident) have been reported from clinical laboratories throughout Alaska to the CDC’s Arctic Investigations Program (AIP) in Anchorage. Isolates are sent to AIP, where identification, capsular serotyping, and antimicrobial susceptibility testing are performed using standard methods (3). Annually, participating laboratories compare their records with a list of isolates received by AIP and report any missing cases. This case review has shown that AIP receives isolates from at least 90% of cases (3). For this study, we reviewed IPD surveillance data from 1986 to 2009.
Carriage studies. Yearly surveys for nasopharyngeal (NP) carriage of S. pneumoniae were performed to evaluate the development of antimicrobial resistance and the impact of the seven-valent pneumococcal conjugate vaccine in several Alaska populations (14, 15). These included NP swabs collected from (i) Anchorage (approximately 450 children <5 years old/year, from 2000 to 2004 and 2008 to 2009), (ii) region A, which includes one village in which 2 outbreak cases occurred (approximately 800 persons of all ages, from 1998 to 2004 and 2009), (iii) region B (approximately 2,000 persons of all ages each year, in 2008 and 2009), and (iv) region C (approximately 1,000 persons of all ages each year in 1998 to 2004 and 2008 to 2009). All participants provided written informed consent. The study was approved by the ethics review boards of the Centers for Disease Control and Prevention (Atlanta, GA), the Alaska Native Tribal Health Consortium (Anchorage, AK), the Norton Sound Regional Health Corporation (Nome, AK), the Bristol Bay Regional Health Corporation (Dillingham, AK), and the Yukon Kuskokwim Delta Health Corporation (Bethel, AK). For each survey, an NP swab was either plated directly on selective medium (1998 to 2004) or placed in skim milk, tryptone soy broth, glycerol, and glucose (STGG) transport medium (2008 to 2009) for transportation to AIP for isolation of pneumococci and characterization of isolates. Isolates were serotyped by standard Quellung methods.

Genetic subtyping. Pulsed-field gel electrophoresis (PFGE) was performed on all 12F IPD isolates and all carriage isolates from each outbreak region. Further genetic subtyping, multilocus sequence typing (MLST), and multilocus boxB sequence typing (MLBT) were performed on selected 12F isolates that were representative of the different PFGE patterns.

Pulsed-field gel electrophoresis. DNA-containing agarose blocks were prepared as previously described (16). Agarose blocks were incubated in lysis buffer (0.2 M NaCl, 0.01 M EDTA, 0.5% deoxycholate, 0.5% Brij 58, and 6 mM Tris-HCl [pH 8.0]) supplemented with 10 mg/ml lysozyme at 37°C for 3 h followed by incubation in ES buffer (0.5 M EDTA, 34 mM N-lauroyl sarcosine) supplemented with 10 mg/ml proteinase K at 50°C overnight. Blocks were washed 6 times with Tris-EDTA buffer (0.01 M Tris-HCl, 1 mM EDTA [pH 8.0]); gel plugs were cut from the blocks and digested for 12 to 18 h with 25 U Smal at 37°C. Digested DNA plugs were placed in wells of a 1% agarose gel (Sea Kem ME; Lonza, Rockland, ME) prepared in 0.5× Tris-boric acid-EDTA buffer and electrophoresed in a contour-clamped horizontal electric field apparatus (CHEF-DR III; Bio-Rad, Hercules, CA). Electrophoresis conditions included pulse times of 2 to 30 s for 23 h, a voltage of 6 V/cm, a flow rate of 1 liter/min, and a temperature of 14°C. Gels were stained with ethidium bromide, and the images were digitized on a Gel Doc system (Bio-Rad). DNA banding patterns were analyzed with BioNumerics software (version 5.0; Applied Maths, Austin, TX). Interpretation of strain relatedness on the basis of PFGE patterns was done according to accepted criteria (17).

Multilocus sequence typing. MLST was performed on selected 12F IPD and carriage isolates (n = 101) from each predominant PFGE pattern as described previously (18). We determined the sequence types (STs) by comparing the sequences from Alaska isolates with those in the pneumococcal MLST database (http://spneumoniae.mlst.net/).

Multilocus boxB sequence typing. A total of 92 IPD and carriage isolates were further characterized by a new sequence-typing method specifically developed with 12F pneumococci (19). Briefly, BOX elements are short repetitive sequences that occur in intergenic regions throughout the pneumococcal chromosome and often consist of single boxA and boxC modules that flank one or more copies of the 45-bp boxB module. At a given boxB locus (position in the chromosome), boxB can vary between isolates in the number of times it is repeated and in the sequence of its repeats. The typing method is based on PCR followed by sequencing, on both DNA strands, of 10 different boxB loci. Unique boxB sequences at a locus define alleles, and unique combinations of alleles across the 10 loci define boxB sequence types (BTs) in a manner analogous to MLST.

RESULTS Statewide epidemiology of invasive disease. Figure 2 shows the number of cases of 12F IPD by year and region in Alaska from 1986 to 2009. A total of 171 cases of 12F IPD were detected, which represent 5.4% of total IPD (3,178 cases). The largest proportion.

FIG 1 Regions and locations of pneumococcal carriage surveys in Alaska.

FIG 2 IPD serotype 12F cases by region—Alaska, 1986 to 2009.
of cases occurred in the Anchorage region (73 cases, 42.7%). Two broad waves of disease occurred, with statewide rates reaching approximately 3.5 and 2.4 cases/100,000 in 1988 and 2007, respectively. Cases occurred nearly every year in the Anchorage region but only sporadically throughout the rest of the state.

The highest annual rate of 12F disease in the Anchorage region was 2.7 cases/100,000 (1988); the average rate between 1986 and 2009 was 0.8/100,000/year. Maximum rates in middle-sized geographic regions were 9.5 and 7/100,000 (region G in 1987 and region F in 2001), while outbreaks with substantially higher rates of disease occurred in the smaller region E (1987, 60/100,000; total population, 6,000), region A (previously reported outbreak [11], 2003, maximum rate 75/100,000, total population 15,000), and region B (new outbreak, 2007, maximum regional rate 52/100,000). All 13 cases in 2007 occurred in the central area of region B (rate of 150/100,000).

**Recent outbreak.** While 12F cases were identified in region B from the time surveillance began in 1986 through 1989, no cases were identified in region B between 1990 and 2005 (Fig. 2). There were 21 cases in region B between October 2006 and July 2008. The first three cases occurred in small villages far from the main population center, as did the last four. The outbreak peaked in 2007, when cases occurred only in the main population center of the region and the surrounding villages (total population approximately 11,000), with an attack rate of 152/100,000. Most case patients (17/21, 81%) were >30 years old (median age, 44 years); four were <5 years old. Seventeen of 21 case patients had a clinical syndrome of bacteremic pneumonia, and one patient died. Over the surveillance time period, characteristics of cases of 12F IPD in Anchorage and other regions were similar in terms of age, sex, and clinical syndrome to those of cases in region B during the recent outbreak. Case fatality ratios, however, were significantly higher in those regions outside region B and the Anchorage region (13% and 4%, respectively, P = 0.05).

In region B, coverage with the 23-valent polysaccharide vaccine, which includes 12F polysaccharide, was 90% in persons >65 years old in 2008. Five of the six adult case patients >55 years old identified during the 2006 to 2008 outbreak had received at least one dose of the polysaccharide vaccine before disease onset. All four case patients <5 years old had received four doses of the pneumococcal conjugate vaccine, which does not include 12F polysaccharide.

**Carriage data.** As noted previously, carriage studies were performed in 4 regions, including Anchorage. Table 1 shows the proportion of all nasopharyngeal carriers of *S. pneumoniae* who carried serotype 12F strains in region A by year and area village (for other regions, see Table S1 in the supplemental material). In the three rural regions, 61% of village residents participated in the carriage studies over the course of all the study years (annual participation ranged from a low of 37% to a high of 75%). The superscript “a” indicates the years in which cases of 12F IPD were identified among persons of any age in these regions (but not necessarily in the carriage villages). Years in which cases of 12F IPD occurred in carriage study villages are footnoted.

In general, carriage of 12F strains was uncommon. Between 2000 to 2004 and 2008 to 2009, 12F carriage was not identified among 3,043 children <5 years old in Anchorage and no cases of 12F IPD occurred in children during this period, although 12F IPD occurred among Anchorage adults during these years. 12F carriage was identified in <0.5% of carriers of all ages in region C, where only 1 case of 12F IPD occurred in the last 10 years (in 2008, in a village not included in the carriage study). Across all rural regions where carriage studies were performed, 12F carriage was much more common among persons >18 years old than among children <18 years old, occurring in 2.1% and 0.7% of individuals in these age groups, respectively (P < 0.001) (Fig. 3).

Of the villages evaluated for carriage in region A (the site of the 2003–2006 outbreak), only one (village 1) had cases of 12F IPD during a year in which a carriage survey was done. During that year (2003) in this village, 45 12F carriers (18% of the 250 *Streptococcus pneumoniae* carriers) were identified, and 31 of 45 (69%) were <18 years old. Only 5 (29%) of 17 carriers in other villages in region A without coincident IPD were <18 years old (P = 0.02). The following year, in village 1, after the peak of the outbreak, only 2 (22%) of 9 12F carriers were <18 years old (P = 0.02, Fisher’s exact test). In 2009, 3 years after the last 12F case in region A, no 12F carriage was found in village 1. Similarly, in neighboring region B, 2 years after outbreak-associated cases of IPD occurred in village 1, no 12F carriers were identified in persons of any age in that village.

**Identification of outbreak clones.** Genetic subtyping data

![Proportion of 12F carriers by age class—regions A, B, and C.](http://jcm.asm.org/Downloaded-fromhttp://jcm.asm.org/)
from isolates collected between 1998 and 2008 are presented in Table 2. Table 2 lists the PFGE, MLST, and MLBT types and numbers of isolates. Note that all PFGE type B’s were either ST218 or ST220 by MLST. All PFGE type A’s were ST1527, and the single PFGE type C was ST989 by MLST.

PFGE type A/ST1527 did not appear in Alaska until 2003 (Table 2), where it caused the first of the two recent outbreaks. All carriage isolates in region A from 2003 to 2004 were also of PFGE type A/ST1527 (data not shown). PFGE type A/ST1527 was not present in over 100 12F IPD isolates from Alaska prior to 2003, but it subsequently appeared in Anchorage, where it remained a cause of IPD between 2004 and 2008 (Table 2). MLBT resolved four different boxB types among otherwise indistinguishable ST1527 isolates from the 2003 to 2006 outbreak in region A and another type from Anchorage. The predominant boxB type from this outbreak was BT88, with 6 isolates (Table 2).

The outbreak in region B was caused mostly by PFGE type B/ST218. All carriage isolates from region B were also ST218. Isolates of this clone that are indistinguishable by PFGE and MLST were present in Alaska as far back as 1998 (Table 2). In fact, PFGE type B/ST218, and variants thereof, were the predominant 12F isolates in Alaska prior to 1998 (data not shown). Subtyping by MLBT revealed a predominance of a single boxB type, BT5, from the 2006 to 2008 outbreak in region B. The first appearance in Alaska of this BT was in cases in region F and Anchorage in 2001. This BT was also found among invasive isolates from California in 1995 and 1996 (19), indicating its broader geographic distribution.

**DISCUSSION**

Evaluation of 23 years of surveillance data from Alaska shows two broad waves of 12F IPD. While disease occurred at low, relatively stable levels in the large population center of Anchorage, the peaks observed in the state-wide data are due primarily to outbreaks of disease in less heavily populated rural areas. This pattern might be the result of waning population immunity in small isolated populations in which the outbreak strain disappears from circulation rapidly, in contrast to more stable immunity in the larger population in Anchorage, where a low level of IPD was continuously present. The outbreak in region A initially appeared to be temporally associated with the subsequent outbreak in region B, but subtyping data suggest otherwise. While the region A clone (PFGE type A/ST1527/BT88 and PFGE type B/ST218/BT5) differ considerably from each other at the genetic level. For example, they differ at all 7 loci used for MLST and at 8 of 10 boxB loci used for MLBT and therefore belong to distinct clonal complexes.

**Table 2**

<table>
<thead>
<tr>
<th>Year</th>
<th>Anchorage</th>
<th>Region A</th>
<th>Region B</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006-2008</td>
<td>13</td>
<td>C (1)</td>
<td>969</td>
</tr>
<tr>
<td>2003-2005</td>
<td>10</td>
<td>B (9)</td>
<td>218 (6), ND (4)</td>
</tr>
<tr>
<td>2006-2008</td>
<td>13</td>
<td>B (9)</td>
<td>218 (6), ND (4)</td>
</tr>
<tr>
<td>2006-2008</td>
<td>2</td>
<td>A (2)</td>
<td>ND (2)</td>
</tr>
<tr>
<td>2006-2008</td>
<td>21</td>
<td>B (20), ND (1)</td>
<td>218 (18), 220 (1)</td>
</tr>
<tr>
<td>1998-2002</td>
<td>11</td>
<td>B (10)</td>
<td>218 (6), ND (4)</td>
</tr>
<tr>
<td>1998-2002</td>
<td>11</td>
<td>B (10)</td>
<td>218 (6), ND (4)</td>
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<tr>
<td>1998-2002</td>
<td>11</td>
<td>B (10)</td>
<td>218 (6), ND (4)</td>
</tr>
<tr>
<td>1998-2002</td>
<td>11</td>
<td>B (10)</td>
<td>218 (6), ND (4)</td>
</tr>
</tbody>
</table>

Note: Not all isolates were typed by MLST and MLBT, for each PFGE subtype, ST, or BT, the number of isolates of that type is in parentheses.

**Columns list the total number of 12F IPD isolates in that site by year, and then the number of strains in each PFGE subtype, followed by MLST and MLBT results among each PFGE subtype. (Note that not all isolates were typed by MLST and MLBT.)**

**ND, not done.**
during the postoutbreak period in these rural areas and then might be reseeded from reservoirs in larger population centers. Data from the rural villages in regions A and B show that 12F carriage in adults is generally low, but much more common, except in outbreak settings. Persistent 12F IPD in adults in Anchorage further suggests a reservoir in this population, although we did not evaluate carriage in this group. Substantial carriage in persons <18 years old (and especially those <5 years old) was found only during the first year of the outbreak in the only village we were able to evaluate during a 12F outbreak. A similar trend was seen in a description of a serotype 1 outbreak in rural Alaska, where carriage among young children peaked early in the outbreak and then declined (8). Thus, the onset of the outbreak was accompanied by an inversion of the usual carriage pattern for this serotype—instead of predominance in adults, most carriage was in children (who were presumably immunologically naïve).

Our data suggest that 12F epidemiology in rural Alaska is characterized by (i) introduction of the organism into an immunologically naïve population, (ii) widespread transmission of the organism, with the appearance of IPD cases, (iii) rapid development of widespread mucosal immunity, with the elimination of carriage and transmission, and then the disappearance of IPD, and (iv) regrowth, through incoming birth cohorts, of an immunologically naïve population, which sets the stage for reinitiation of the cycle with the next introduction of 12F, most likely from larger populations in which its circulation was sustained. This cycle might occur with other pneumococcal serotypes but might be more obvious with 12F, which classically results in IPD but is rarely carried, possibly due to some combination of an enhanced human immune response to the 12F antigen or a 12F-specific tendency for short carriage periods and limited transmission. As shown in region A, village 1, adults became the predominant reservoir of the organism within 1 year as the overall 12F carriage rate decreased. In larger population centers, such as Anchorage, outbreaks of 12F disease were not observed, and IPD occurred primarily in adults. We evaluated but did not document carriage in children in Anchorage and did not evaluate carriage in adults in this population. It is possible that in larger communities, continuous exposure to 12F does not allow the development of a cohort of immunologically naïve children and the population conditions for an outbreak are more difficult to meet.

While our data provide clarification of the recent epidemiology of 12F disease in Alaska, community-wide outbreaks of pneumococcal disease are uncommon, and the appropriate public health response has not been well defined. The multiple BTs found from this outbreak are consistent with ongoing transmission (i.e., growth and mutation) of the bacteria in a community over a period of time and contrasts with the indistinguishable BTs from the institutional outbreaks of 12F disease identified previously in Texas (1989) (22), Maryland (1992 with a single exception) (23), and California (2004) (19, 24). Most pneumococcal outbreaks reported in the recent literature were short lived (1 to 2 months) and occurred among closed or institutional populations such as day care or military groups (7). Three previous reports of 12F outbreaks fit this pattern, occurring in a day care center (23), a jail (22), and a homeless shelter (24). In contrast, there is less experience with documented community-wide outbreaks of pneumococcal disease, such as we observed in rural Alaska, which persisted for prolonged periods (1 to 3 years). Serotype 1 and serotype 5 outbreaks in populations with some characteristics in common with rural Alaska have been reported from Australia (6), northern Canada (10, 25), and Israel (26), but we are aware of only one other report of a community-wide 12F outbreak (9). In this outbreak, 17 cases occurred over a 21-month period and recommendations for increased use of the polysaccharide vaccine were made. While antimicrobial chemoprophylaxis is likely to be useful in the management of small, short-lived, closed-population outbreaks (27), vaccine options might be a more useful alternative in community outbreaks, which are likely to be of longer duration and involve larger populations (7, 28). The 23-valent pneumococcal vaccine has reasonable efficacy against 12F IPD in healthy adults but is not likely to be useful for infants or the very elderly (29, 30). Information on current levels of 12F disease might be important for encouraging immunization with polysaccharide vaccine, but prevention options for the very old and the very young are still limited.

Our study has several limitations. While we have IPD surveillance data obtained continuously from 1986, carriage data obtained continuously on relevant populations were not available. We were fortunate to have carriage data available for some populations in which outbreak IPD was actively occurring. We did not assess carriage in adults in Anchorage (presumably the reservoir for 12F), since we did not enroll adults there but had carriage data only for children <5 years old.

Consistent with other observations, 12F strains are rarely identified in routine carriage surveys but are well documented to cause outbreaks of serious IPD. 12F IPD in Alaska is characterized by episodic outbreaks in isolated rural areas, with subsequent disappearance of the pathogen. The onset of outbreak disease is accompanied by a reversal of the usual adult carriage pattern to widespread, but short-lived, carriage among children. Rural Alaska appears to be particularly prone to 12F outbreak activity, and we hypothesize that this is in part due to the small, isolated structure of its population—12F carriage is introduced, spreads rapidly among immunologically naïve cohorts, and then is eliminated rapidly as herd immunity develops. This pattern is less likely to be observed in larger, less isolated populations, where, due to the continued circulation of 12F strains, development of truly naïve populations does not occur. 12F appears to represent one end of the spectrum of behavior of S. pneumoniae strains—a virulent strain whose presence in the nasopharynx is limited by its own immunogenic/ transmission characteristics. The behavior of 12F in rural Alaska highlights characteristics of the residents that might contribute to high rates of IPD in this population.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

REFERENCES


