Evidence for Transmission of Pertussis in Schools, Massachusetts, 1996: Epidemiologic Data Supported by Pulsed-Field Gel Electrophoresis Studies

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In 1996, 18 of 20 pertussis outbreaks reported in Massachusetts occurred in schools. Pertussis surveillance data were reviewed and a retrospective cohort study was conducted in a high school that experienced an outbreak. *Bordetella pertussis* isolates from 9 school cases and from 58 cases statewide were examined by use of pulsed-field gel electrophoresis (PFGE). Statewide incidence rates were highest among children aged <1 year, 10–14 years, and 15–19 years (106, 117, and 104 cases per 100,000, respectively). Among 34 confirmed and 20 probable cases at the school, 61% had cough onset within 8 weeks of school opening. Five different PFGE types were identified among the 58 *B. pertussis* isolates from throughout the state. All 9 isolates from the affected high school were the same PFGE type. School-aged children may play an important role in pertussis epidemics. Consideration should be given to use of acellular pertussis vaccines among school-aged children.

Although pertussis has been well controlled for nearly 50 years in the United States by routine vaccination of children aged <7 years, *Bordetella pertussis* remains endemic, and periodic epidemics continue to occur every 3–4 years [1]. In 1996 the United States experienced increased pertussis activity: 7796 cases of pertussis were reported, an increase of 18% over the previous peak in 1993 [2]. Large outbreaks were reported in Massachusetts, Vermont, Washington, Iowa, Kentucky, and New Hampshire [3]. In recent years there has been a change in the epidemiology of reported cases of pertussis, with a shift toward older age groups. During 1994–1996, the average reported incidence of pertussis in the United States among persons aged 10–19 years increased by 106%, compared with 1990–1993 data [2]. Possible reasons for this increase among adolescents include faster waning of vaccine-induced immunity and improved clinical and laboratory recognition of pertussis in this age group [4, 5].

Twenty distinct outbreaks of pertussis were reported in Massachusetts in 1996, 18 of which were in schools. The high culture isolation rate observed in these outbreaks provided an opportunity to evaluate the use of pulsed-field gel electrophoresis (PFGE) techniques to study patterns of pertussis transmission. PFGE methods have been found to be useful in the study of pertussis transmission in Canada [6] but have not, as yet, been evaluated thoroughly in the United States. The objectives of our study were as follows: (1) to describe the epidemiology of pertussis in Massachusetts in 1996; (2) to determine the extent of pertussis transmission in schools; and (3) to assess the role of school-aged students in periodic outbreaks of pertussis.

**Methods**

**Surveillance for pertussis.** In Massachusetts, cases were identified by physicians, school nurses, and other health care providers. School nurses referred suspected pertussis cases to their physician, who obtained a nasopharyngeal swab or drew blood for serologic analysis. Laboratory confirmation of *B. pertussis* was based on the following: (1) isolation of the organism from nasopharyngeal specimens; or (2) for persons aged ≥11 years, a single serum specimen with an anti-pertussis toxin IgG antibody concentration ≥20 μg/mL, as assayed by ELISA at the Massachusetts State Laboratory Institute (SLI) [7]. Both culture and serologic analysis are available free of charge through the Massachusetts Department of Public Health (MDPH). The majority of pertussis cases were reported to MDPH epidemiologists by the SLI on the basis of positive culture and/or results of serologic testing.

The clinical case definition of pertussis for outbreak settings was a cough illness lasting at least 2 weeks without other apparent cause as reported by a health professional [8]. A confirmed case was defined by positive serology or isolation of *B. pertussis* or if it met the clinical case definition and was supported by epidemiologic linkage to a laboratory-confirmed case. A probable case met the clinical case definition without laboratory confirmation or epidemiologic linkage. Epidemiologic linkage was defined as onset of
disease within 7–28 days after contact with a laboratory-confirmed case.

National pertussis surveillance data derive from investigation of suspected pertussis cases by field staff at state and county health departments. The data are reported to the Centers for Disease Control and Prevention (CDC) through the National Notifiable Diseases Surveillance System. This system collects information on age, diphtheria, tetanus, and pertussis (DTP) vaccination history, clinical characteristics (date of onset and duration of cough and the presence of paroxysmal cough, whoop, vomiting, cyanosis, apnea, pneumonia, seizures, encephalopathy, or death), hospital admission, antimicrobial therapy, and results of laboratory testing for B. pertussis.

Retrospective cohort study. A retrospective cohort study was conducted between 5 January and 7 February 1997 in a high school with an enrollment of 1003 students that had experienced an outbreak of pertussis. The index case had onset of cough on 6 June 1996, and cases occurring between this date and December 31 were included in the investigation, although the outbreak continued into 1997. Confirmed and probable cases were interviewed in person by a study investigator to obtain a detailed history of contacts. The immunization records of all students attending the school were reviewed for the number of doses and the date of their last pertussis-containing vaccination. Because of reports of pertussis in members of sports teams, line listings of members of all sports teams in the school were obtained. Investigation and data collection were carried out in accordance with MDPH's routine pertussis surveillance protocols and its confidentiality policies.

PFGE typing of B. pertussis isolates. Isolates from 9 of the 12 culture-positive cases at the affected high school were available for PFGE testing. In addition, 58 of 216 isolates collected in Massachusetts during 1996 were tested. These 58 isolates were selected based on geographic diversity. PFGE testing of B. pertussis strains was done at both the Massachusetts SLI and the National Center for Infectious Diseases, CDC, by use of similar protocols. The PFGE patterns of the Massachusetts isolates were compared with 235 isolates banked at CDC, which were obtained from 21 states between 1985 and 1997.

The 67 B. pertussis isolates were incubated for 3 days at 37°C on Bordet-Gengou agar plates. One loopful of growth was harvested and suspended in 1.0 mL of pH 8.0 SE buffer (75 mM NaCl, 25 mM EDTA), washed twice with SE buffer at room temperature, and processed as described elsewhere by de Moissac et al. [6]. Two segments were cut from each plug, and PFGE was done after restriction with XmlI on 1 segment and SpeI on the other (New England Biolabs, Beverly, MA). The gels were run by use of a contour-clamped homogeneous electric field mapper (Bio-Rad Laboratories, Hercules, CA) at 14°C by use of TBE running buffer (10.9 g Tris, 6.0 g boric acid, 14 mL 0.5 M EDTA, and distilled water to make 1.0 L, pH 8.0). PFGE was run for 22 h with initial and final switch times of 2.2 and 54.2 s, respectively. Strains were considered to be genetically indistinguishable if their restriction pattern profiles had the same number of bands and the corresponding bands were of the same apparent sizes.

Statistical methods. Analyses of the cohort study were done by use of EPI INFO 6.1 (Centers for Disease Control and Prevention, Atlanta). Gels displaying DNA fragment patterns were electronically scanned directly into a computer via a charge-coupled device video camera directly connected to a personal computer. Acquired images were compared both visually and with the assistance of analysis software (Molecular Analyst Fingerprinting Plus by Bio-Rad) to determine relatedness.

Results

Of 7796 pertussis cases reported nationwide in 1996, 1245 cases (16%) were reported from Massachusetts. Of the Massachusetts cases, 356 (28%) were confirmed by isolation of B. pertussis, 698 (55%) were confirmed serologically, and 211 (17%) were confirmed by epidemiologic linkage to a laboratory-confirmed case. The peak number of cases in Massachusetts occurred in November, and 524 (42%) had cough onset during September–November (figure 1). When compared with the United States as a whole, the age-specific incidence rates in Massachusetts were higher among those aged <1, 10–14, 15–19, and ≥20 years (table 1). In Massachusetts, 67% of cases were aged 10–19 years, compared with 26% in the same age group nationally. Of 833 cases aged 10–19 years in Massachusetts, 216 (26%) were confirmed by isolation of B. pertussis, and 503 (61%) were confirmed by serologic testing.

Retrospective cohort study. There were 54 cases of pertussis among students at the affected high school, an attack rate of 5.4%. Thirty-four cases (63%) were classified as confirmed: of these, 12 (35%) were confirmed by culture, 12 (35%) by serologic testing, and 10 (30%) by epidemiologic linkage to a laboratory-confirmed case. There were 20 (37%) probable cases, 19 of which had at least 1 other symptom of pertussis (whoop, paroxysm, or posttussive vomiting) in addition to cough for ≥14 days. The first case identified among students had cough onset on June 6, and the last case in the time frame of the study had cough onset on 26 December 1996 (figure 1). Thirteen cases (24%) had cough onset before school opened on September 3. Thirty-five of the cases (65%) did not report having contact with a case. Of the 19 cases who reported contact with a possible case, 15 of the contacts were students at the school and 13 (68%) of these were known cases. Peak incidence of pertussis at the affected high school was in September and October, whereas peak incidence statewide was 1 month later (figure 1).

Disease among school students was moderately severe; 48 (89%) reported paroxysmal cough, 35 (65%) had a duration of cough ≥30 days, 25 (46%) reported posttussive vomiting, 35% had visited a physician at least twice, and 27% reported a whoop. Two cases developed radiologically confirmed pneumonia.

Students at the school ranged in age from 13–19 years and were highly vaccinated (95% had received ≥4 doses of pertussis-containing vaccine in childhood). There was no significant difference in age, number of doses of pertussis-containing vaccine received, or time since last vaccination among cases compared with noncases (table 2). There was a significantly higher rate of disease among girls compared with boys (P = .04). Attack
rates in grades 9, 10, 11, and 12 were 3.9%, 4.0%, 6.0%, and 2.7%, respectively.

The attack rate among all students who were members of small sports teams (≤15 players) was 15.6%, compared with 3.3% for those who were members of large teams (>15 players) and 3.4% for those who were not members of a sports team. Students who participated on small sports teams that had at least 1 case member had a 2.9 times greater risk of disease compared with those on large sports teams with at least 1 case member (95% confidence interval, 1.01–8.1). There was no significant increase in risk of disease among students who were members of large sports teams that had at least 1 case member, compared with students who did not belong to any sports team.

**PFGE types.** Restriction profiles of all 67 isolates, 9 from the affected high school and 58 from throughout the state, yielded 16–20 fragments ranging in size from ∼40 to 650 kbp, which were grouped into 5 PFGE patterns (figure 2). Thirty-five isolates (60%) were of Massachusetts PFGE (MAPFGE) type 1; 8 (13%) were MAPFGE type 2; 11 (19%) were MAPFGE type 3; 2 (3%) were MAPFGE type 4; and 2 (3%) were MAPFGE type 5. All 9 isolates from cases at the affected high school had the same PFGE pattern (MAPFGE type 1).

### Table 1. Age distribution and incidence of reported pertussis cases, United States and Massachusetts, 1996.

<table>
<thead>
<tr>
<th>Age groupa (y)</th>
<th>United States</th>
<th>Massachusetts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Incidence rateb</td>
</tr>
<tr>
<td>&lt;1</td>
<td>2368 (31)</td>
<td>61.5</td>
</tr>
<tr>
<td>1–4</td>
<td>1096 (14)</td>
<td>7.1</td>
</tr>
<tr>
<td>5–9</td>
<td>864 (11)</td>
<td>4.4</td>
</tr>
<tr>
<td>10–14</td>
<td>1280 (16)</td>
<td>6.7</td>
</tr>
<tr>
<td>15–19</td>
<td>750 (10)</td>
<td>4.0</td>
</tr>
<tr>
<td>&gt;20</td>
<td>1413 (18)</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>7771 (100)</td>
<td>2.9</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) except as indicated. Reported through National Notifiable Diseases Surveillance System.

a Cases where age is unknown are excluded.
b Per 100,000 population.

Clustering of PFGE types was observed in several other small outbreaks. There were 6 MAPFGE type 1 isolates from another high school and 3 MAPFGE type 2 isolates from members of a swim team from an additional high school. One high school had 2 different PFGE types circulating: 18 MAPFGE type 1 isolates and 11 MAPFGE type 3 isolates. MAPFGE type 1 isolates were obtained from 4 household case-pairs, including...
a mother and son and a mother and daughter. MAPFGE type 2 isolates were obtained from 2 household case-pairs, one from twins and the other a father and son.

MAPFGE type 1 was also the predominant pattern among 235 isolates collected from 21 states in the United States during 1985–1997. Among the US isolates, 87 (37%) were MAPFGE type 1, 13 (6%) were MAPFGE type 6, and 11 (5%) were MAPFGE type 2. The remaining PFGE types in the United States database (which appeared in proportions ranging from 0.4% to 26%) were not seen in Massachusetts during 1996. MAPFGE type 1 has been documented in the United States since 1989 and has been the predominant strain throughout the study period. To date, the only other report of MAPFGE type 4 has been from Cincinnati in 1993, also isolated during an outbreak.

Discussion

Since the early 1980s, enhanced surveillance measures in Massachusetts (including the availability of a reliable serologic test) have been associated with a higher reported incidence of pertussis relative to the United States [7]. In 1996, this difference was most noticeable among persons aged ≥10 years and suggests that Massachusetts surveillance data may represent a more accurate picture of the true age-specific burden of pertussis disease than that represented by aggregate data from the United States. The difference between incidence rates among children aged <1 year in Massachusetts, compared with the United States, was less marked. The following year (1997), pertussis incidence in infants was less than in the United States as a whole (33.7 vs. 52.1 per 100,000). Thus, the higher incidence in 1996 may have been due to the fact that it was an epidemic year in Massachusetts.

In Massachusetts in 1996, coverage with at least 3 doses of pertussis-containing vaccine was 98% for children aged 19–35 months. Coverage with ≥4 doses was 88% [9]. Prior to October 1996, all children in the state received whole-cell pertussis vaccine manufactured by Massachusetts Biologic Laboratories. Safety and immunogenicity studies have demonstrated that Massachusetts’ whole-cell vaccine is both safe and immunogenic [10]. A lower antibody response to pertussis toxin antigen has been noted in recipients of Massachusetts vaccine compared with Lederle whole-cell DTP [10]. However, higher antibody responses to filamentous hemagglutinin and pertactin were noted in recipients of Massachusetts vaccine compared, with the Lederle vaccine. The significance of these findings is unclear, as no correlation has been demonstrated between circulating levels of these antibodies and clinical protection against disease. Although pertussis incidence was high among adolescents, it was not appreciably higher among Massachusetts children in the 1–4 or 5–9 year-old age groups than in the United States. This suggests that the Massachusetts immunization program was effective in protecting children aged 1–10 years against pertussis.

In the cohort study at the affected high school, vaccination rates were high, reflecting the high immunization coverage in the state. Thus, outbreaks of pertussis can occur among high school students previously vaccinated in early childhood. The most likely cause of such outbreaks is waning immunity [11, 12]. Our study did not find an increase in attack rate associated with older age, grade, or greater number of years since last vaccination, as might be expected with waning immunity. However, patterns of interaction among students of different ages or in different grades were unknown, and previous pertussis may have contributed to the lower attack rate observed among 12th-grade students.

Although pertussis outbreaks have been reported among school-aged children, there is a lack of information on which to base outbreak control measures [13]. In this study, participation on a small sports team that had at least 1 member identified as a case was associated with an increased risk of disease, whereas participation on a large sports team with a case was not. This may be because the opportunity for close contact with an infected team member is greater on a small team than on a larger team. However, cases among smaller teams may also be more readily identified than cases on larger teams. Further studies are needed to assist in targeting prophylactic measures in school outbreaks.

PFGE studies demonstrated that at least 5 distinct strains of B. pertussis were circulating in Massachusetts in 1996, suggesting that numerous reservoirs of B. pertussis existed. All 9 isolates from the affected high school were MAPFGE type 1. Strains obtained from 2 other high schools also had the same PFGE type identified. However, in one high school, where 29 strains were tested, 2 different PFGE types were identified. This may be explained by 2 separate introductions into the school, with subsequent transmission of both types. The uniformity of PFGE types within epidemiologically linked clusters in our study is similar to that seen in Canada [6] and suggests that PFGE typing may be a valuable tool for identifying reservoirs of pertussis and studying transmission patterns.

Table 2. Selected characteristics of 54 pertussis cases and 943 noncases at a Massachusetts high school in 1996.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Noncases</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD, y)</td>
<td>15.9 ± 1.1</td>
<td>15.7 ± 1.2</td>
<td>.30</td>
</tr>
<tr>
<td>≥4 doses* DTP</td>
<td>52 (96)</td>
<td>898 (95)</td>
<td>.77</td>
</tr>
<tr>
<td>TSLD ‡ (mean ± SE)</td>
<td>11.9 ± 1.9</td>
<td>11.7 ± 2.1</td>
<td>.57</td>
</tr>
<tr>
<td>Sex* (% female)</td>
<td>34 (63)</td>
<td>450 (48)</td>
<td>.04</td>
</tr>
<tr>
<td>Participateda on any sports team</td>
<td>19 (46)</td>
<td>337 (36)</td>
<td>.38</td>
</tr>
<tr>
<td>Small team ae participant</td>
<td>10 (24)</td>
<td>54 (6)</td>
<td>.0001</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) except as indicated. DTP, diphtheria, tetanus, and pertussis.

* No. of doses was unknown for 3 noncases.
‡ Time since last dose of pertussis vaccine.
¢ Sex was unknown for 20 noncases.
§ Only cases occurring during school year are included.
e Team with <15 participants.
Our study has several limitations. Variability in methods used for diagnosis of pertussis in different states and general lack of awareness of pertussis as a possible cause of cough illness among adolescents and young adults complicate interpretation of differences between Massachusetts and national pertussis surveillance data. The retrospective nature of the cohort study in the affected high school resulted in limited exposure histories.

Despite these limitations, several lines of evidence suggest that pertussis transmission occurred in schools in Massachusetts in 1996 and that school-aged children, particularly those aged 10–19 years, played an important role in the statewide outbreak. There was a temporal association between school opening and a peak in pertussis cases among older school-age children 2–3 months later. Similar peaks in pertussis incidence can be seen in Massachusetts in the fall of 1995 and 1997 (figure 1). Among cases at the affected high school, a school friend was the most likely source of pertussis infection. Although at least 5 different PFGE types were circulating in Massachusetts in 1996, all 9 available *B. pertussis* isolates from cases at the affected high school were PFGE type 1. At 3 other high schools, PFGE types were found to be the same among students from the same school, suggesting person-to-person transmission within the school.

Further studies of the role of adolescents and young adults in periodic pertussis outbreaks and their ability to transmit pertussis to unvaccinated young infants are required. Enhanced surveillance for pertussis among high school students should be conducted in other states to determine the generalizability of the Massachusetts experience. Clinical trials are currently underway to determine the safety and efficacy of acellular pertussis vaccine in adolescents and adults. If additional epidemiologic studies confirm our findings in Massachusetts, consideration may be given to licensure and routine use of acellular pertussis vaccines among these older age groups.

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References

10. Steinhoff MC, Reed G, Decker MD, et al. A randomized comparison of...