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Pertussis Outbreak, Southeastern Minnesota, 2012

Alexander G. Theofiles, MD, **Scott A. Cunningham, MS**, **Nicholas Chia, PhD**, **Patricio R. Jeraldo, PhD**, **Daniel J. Quest, PhD**, **Jayawant N. Mandrekar, PhD**, and **Robin Patel, MD**

Department of Medicine (A.G.T.), Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology (S.A.C., R.P.), Department of Surgery (N.C., P.R.J.), Center for Individualized Medicine (N.C., D.J.Q.), Department of Health Sciences Research (J.N.M.), and Division of Infectious Diseases (R.P.), Mayo Clinic, Rochester, MN; and Institute for Genome Biology, University of Illinois at Urbana-Champaign, Urbana (P.R.J.).

Abstract

Objective—To describe clinical and laboratory findings from the 2012 southeastern Minnesota pertussis outbreak.

Patients and Methods—Patients were selected for 2 parts of the study. In the first part, nasopharyngeal swabs from a convenience sample of 265 unique patients were used for both the clinician-requested polymerase chain reaction (PCR) test and culture. B pertussis isolates were tested for macrolide susceptibility and typed using whole genome sequencing and pulsed-field gel electrophoresis. Pertactin gene sequences were analyzed to identify pertactin-deficient B pertussis. In the second part, all patients seen at Mayo Clinic in Rochester, Minnesota, who had PCR results positive for Bordetella pertussis or *Bordetella parapertussis* between January 1, 2012, and December 31, 2012, were analyzed for patient demographic features and vaccination records.

Results—One hundred sixty patients had results positive for *B pertussis*, and 21 patients had results positive for *B parapertussis*. Among the 265 swabs cultured, *B pertussis* was detected by both culture and PCR in 11. One swab was positive for *B pertussis* by culture alone, and 13 were positive by PCR alone. Polymerase chain reaction detected *B pertussis* more frequently than did culture (P=.001). No macrolide resistance was detected. All 12 isolates tested had an altered pertactin gene, including 9 with a signal sequence deletion, 2 with insertion sequence disruptions, and 1 with a premature stop codon. Nine and 3 isolates were pertactin types *prn1* and *prn2*, respectively. Whole genome sequencing and pulsed-field gel electrophoresis detected the presence of multiple *B pertussis* strains. The mean age of patients with pertussis was younger than that of those without pertussis (15.6 and 25.5 years, respectively; P=.002). Compared with those whose test results were negative for *B pertussis*, fewer patients with positive results had received whole-

Correspondence: Address to Robin Patel, MD, Division of Clinical Microbiology, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (patel.robin@mayo.edu)..

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SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mayoclinicproceedings.org.

cell pertussis vaccine (*P*=.02). In the subgroup who had received acellular vaccine exclusively, the time since the most recent pertussis vaccination in those with results positive for *B pertussis* was longer than that in those with negative results (1363 vs 1010 days; *P*=.004).

Conclusion—The 2012 pertussis outbreak in southeastern Minnesota included multiple strains of *B pertussis*, all putatively lacking pertactin. Our findings may indicate decreased efficacy of (and waning immunity from) acellular vaccines as contributors to the outbreak.

Pertussis is a respiratory illness that begins with upper respiratory tract symptoms, progresses to severe paroxysms of cough, and evolves into a convalescent stage. Although infection can be associated with mild symptoms in adults and older children, neonatal infections may be deadly. The etiologic agent is *Bordetella pertussis*. Other less common Bordetella species (*Bordetella parapertussis*, *Bordetella bronchiseptica*, and *Bordetella holmesii*) have been implicated in similar illness. *B pertussis* is found exclusively in humans, with adolescents and adults likely serving as a source of infection of younger children and infants. Since the introduction of vaccines against *B pertussis*, the incidence of pertussis has declined, reaching a nadir in 1976.[1,2] The yearly incidence of pertussis has steadily increased since the 1980s, and communities throughout the United States have experienced a resurgence of pertussis in recent years.[2-5]

Whole-cell pertussis vaccines (thermally or chemically inactivated *B pertussis* cells) were introduced in the 1940s and later combined with diphtheria and tetanus toxoids to form the "DTP" (diphtheria and tetanus toxoids and pertussis) vaccine.[1] Although the vaccines were efficacious and immunogenic, tolerability was limited by vaccine reactions, including local reactions, fever, and febrile seizures. Acellular vaccines, composed of proteins purified from *B pertussis* cell lysates, were introduced in the 1990s. Compared with whole-cell vaccines, acellular vaccines have fewer adverse events.[1,6] Several acellular pertussis vaccines have been used, all of which have contained pertussis toxin, with or without pertactin, filamentous hemagglutinin, and/or fimbrial proteins.[1]

Our medical center is a large, tertiary/quaternary referral center in Rochester, Minnesota, where polymerase chain reaction (PCR) has been used to diagnose pertussis since 1995. Rochester is a city of approximately 109,000 residents located in Olmsted County. In 2012, southeastern Minnesota experienced its largest pertussis outbreak in recent history. That year, Olmsted County reported 237 cases of pertussis (compared with 19 and 28 in 2011 and 2010, respectively). The outbreak occurred in a region with a relatively high vaccination rate compared with that reported in other studies[7]; children in Olmsted County have an 88% rate of acellular pertussis vaccination, higher than the state average of 77%.[8] Herein, we report the epidemiology and clinical and microbiological characteristics of the 2012 pertussis outbreak in southeastern Minnesota and examine possible contributing factors.

PATIENTS AND METHODS

This study was approved by the Mayo Clinic Institutional Review Board. Patients were selected for 2 parts of the study (Figure 1). In the first part, detailed subsequently, a convenience sample of submitted nasopharyngeal swabs was used for both a clinician requested PCR test and an additional culture as part of the study. In the second part, the

electronic charts of all patients seen at the Mayo Clinic in Rochester who had PCR results positive for *B pertussis* or *B parapertussis* between January 1, 2012, and December 31, 2012, were analyzed for patient demographic features and vaccination records. We defined a pertussis or parapertussis case as any patient with PCR or culture positive for *B pertussis* or *B parapertussis*. All patients were cross-referenced with the Minnesota research authorization status database and excluded if records indicated a request to be excluded from research studies.

Culture and *B pertussis* Identification

Nasopharyngeal swabs received for *Bordetella* PCR testing were cultured on Regan-Lowe charcoal media with cephalexin (Hardy Diagnostics). Colonies suspected to represent *B pertussis* were subjected to matrix-assisted laser desorption ionization time-of-flight mass spectrometry,[9] with identification confirmed phenotypically. *B pertussis* isolates were frozen in *Brucella* broth on freezer beads (Hardy Diagnostics).

Real-time PCR for *B pertussis* and *B parapertussis*

Nasopharyngeal swab samples were placed into LightCycler Advanced lysis tubes (Roche Molecular Diagnostics) and subjected to heat lysis on a Thermomixer R (Eppendorf AG) for 6 minutes at 99°C and 1400 rpm, followed by centrifugation for 20 seconds at 20,800*g*. Then 5 μL of the supernatant was combined with 15 μL of PCR master mix and tested using a previously described duplex PCR assay targeting *IS481* and *IS1001* of *B pertussis* and *B parapertussis*, respectively.[10]

Macrolide Resistance Detection in *B pertussis*

Isolates of *B pertussis* were tested for phenotypic and genotypic macrolide resistance. 0.5 McFarland suspensions of each isolate were prepared in normal saline. Using the prepared suspensions, 2 Regan-Lowe agar plates without cephalexin (Hardy Diagnostics) were inoculated for a lawn of growth and allowed to acclimate. A 15-μg erythromycin disk (Becton Dickinson and Company) was placed on one plate and an erythromycin Etest strip (bioMérieux, Inc) on the other. Plates were incubated for 5 days at 35°C in room air. Disk inhibition zone diameters were measured with a micrometer, and Etest minimum inhibitory concentration values were determined following the manufacturer's recommendations. Polymerase chain reaction targeted to the 23S ribosomal RNA gene followed by bidirectional sequencing of the ampli-fied product was performed to detect the A-to-G sequence variation at position 2058 (*Escherichia coli* numbering) associated with macrolide resistance in *B pertussis*.[11] For resistance studies, American Type Culture Collection strains BAA-1335 and 9797 were included as positive and negative controls, respectively.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) of *B pertussis* isolates, including the control isolate *Salmonella* Braenderup H9812 (kindly provided by the Minnesota Department of Health), was performed using XbaI restriction enzyme (Roche Applied Science) as previously described.[12,13] The gel image was captured on a Gel Doc XR system (Bio-Rad Laboratories,

Inc). Analysis of PFGE patterns was performed with GelCompar II software (Applied Maths). Similarity coefficients were calculated using the Dice algorithm.

Whole Genome Sequencing

B pertussis isolates were treated with 5 mg/mL lysozyme and genomic DNA extracted using a Maxwell 16 tissue DNA purification kit (Promega Corporation) and prepared using a genomic DNA Clean & Concentrator-10 kit (Zymo Research Corp). Sequencing of paired-end and Nextera mate-pair libraries was performed using a MiSeq platform (Illumina, Inc) with a 300-cycle kit, resulting in an average coverage of 133X ± 10X genomes per isolate. Reads were processed for adaptor removal and quality filtering using Trimmomatic 0.32 with parameters "ILLUMINACLIP: adapters.fasta LEADING:3 TRAILING:3MAX-INFO: 220:0.1 MINLEN:70."[14] Assemblies were generated using Velvet 1.2.10.[15] Average nucleotide identity was generated using JSpecies.[16] A neighbor-joining tree was calculated and visualized using the APE (Analysis of Phylogenetics and Evolution) package version 3.1-4 in R language.[17] Pertactin gene sequences were identi-fied from open reading frames using Prodigal 2.60[18] alongside manual curation for insertion sequence elements and premature stops. Alignment was performed using MAFFT.[19] Allele typing based on pertactin sequences was done by examination of region 1 repeats.[20]

Medical Record Review and Analysis

A single reviewer (A.G.T.) surveyed the electronic medical record of included patients and collected the following data: age at the time of nasopharyngeal swab collection, date of birth, sex, duration of symptoms before presentation to a health care professional, reported contacts with pertussis, documented symptoms including cough (and whether the cough was productive), nasal symptoms (rhinorrhea or congestion), sore throat, fever, and posttussive emesis. In addition, history of chronic respiratory conditions (asthma or chronic obstructive pulmonary disease) and exposure to tobacco smoke were recorded. Dates of pertussis vaccine administration, age at the time of vaccination, type of vaccine administered (whole-cell killed vs acellular), and influenza vaccination for the 2011-2012 influenza seasons were also recorded for each patient.

With regard to vaccination status, patients were considered "up-to-date" if they had received the correct number of vaccinations at Centers for Disease Control and Prevention (CDC)–defined intervals by 2 weeks (14 days) before the reported date on which symptoms developed and had not passed the last date suggested for a given vaccine without having received it. For example, 12-year-old children would be counted as up-to-date if they had received the 2-month, 4-month, 6-month, 15- to 18-month, and 4- to 6-year acellular vaccines even if the 11- to 12-year-old vaccine had not been administered (as they are not yet 13 years of age). If complete pertussis vaccination records were unavailable in the electronic medical record, available data were collected from the Minnesota Immunization Information Connection. Vaccines were counted as valid if given at an age different from the recommended schedule as long as the CDC-recommended intervals for "catch up" schedules were followed (eg, 1 month between the first and second doses and 6 months between the fourth and fifth doses of acellular vaccine). Vaccinations documented during an era when they were unavailable were corrected to the vaccine type available at the time

documented (eg, a patient documented as having received an acellular vaccine in 1988 was considered to have received a whole-cell vaccine). All patients were included in the final analysis regardless of vaccine record availability.

Statistical Analyses

Descriptive summaries are reported as frequencies and percentages for categorical variables and as mean (SD) and (minimum, maximum) for continuous variables. Comparisons between the groups (eg, results positive for *B pertussis* vs positive for *B parapertussis* or positive for *B pertussis* vs negative for *B pertussis* or *B parapertussis*) were performed using the χ^2 test, Fisher exact test, or Wilcoxon rank sum test, as appropriate. All tests were 2-sided, and $P<.05$ was considered statistically significant.

RESULTS

Study Population

In total, 159 patients (24 from the convenience sample) with positive and 239 patients (all from the convenience sample) with negative PCR test results for *B pertussis* were studied (Table 1). One patient with results positive for *B pertussis* by culture but negative by PCR was also included, as were 21 (1 from the convenience sample) with positive *B parapertussis* PCR test results.

Real-time PCR and Culture

We studied 265 nasopharyngeal swab samples from unique patients in the convenience sample. Culture and PCR detected 11 *B pertussis*– and 1 *B parapertussis*–positive swabs. Culture recovered *B pertussis* in a single sample that was negative by PCR, and PCR detected *IS481* in 13 swabs from which *B pertussis* failed to grow in culture. Polymerase chain reaction detected *B pertussis* more frequently ($N=24$) than did culture ($N=12$) ($P=.001$). Among the PCR- and culture-positive samples, the average real-time PCR crossing point was 29.03 cycles, whereas among the culture-negative samples, the average crossing point was 34.63 cycles ($P=.01$), indicating the presence of more *B pertussis* DNA in the culture-positive than the culture-negative samples.

Macrolide Resistance Detection in *B pertussis*

Neither phenotypic nor genotypic macrolide resistance was detected in any of the 12 *B pertussis* isolates tested. Erythromycin disk diameters were 52 to 64 mm, and minimum inhibitory concentrations were 0.016 to 0.064 μg of erythromycin per milliliter. No mutation at base position 2058 (A-to-G) of the 23S ribosomal RNA gene was detected in any isolate. Together, these data indicate the absence of macrolide resistance.

B pertussis Pertactin Gene Sequencing

Nine and 3 isolates were pertactin types *prn1* and *prn2*, respectively. Based on in silico analysis, all 12 isolates tested had an altered or absent pertactin membrane protein (Table 2). A previously described 84–base pair signal sequence deletion (*prn1*ΔSS) was present in 9 isolates.[21] The pertactin gene was disrupted by *IS481* at nucleotide 1613 in 2 isolates

(*prn2*::*IS481*), the sequences of which closely match those reported by Queenan et al.[22] Finally, a previously described premature stop codon was present at nucleotide 1273 in one isolate.[22,23]

Typing of *B pertussis* Isolates

Whole genome sequencing was performed on all 12 isolates (Figure 2). BP279, BP101, and BP191 clustered together and away from the remainder of the isolates, consistent with their pertactin gene sequences. All but BP191 produced readable PFGE profiles (Figure 3). Pulsed-field gel electrophoresis similarity coefficient values ranged from 55% to 100%; using a similarity cutoff of 90%, there were 4 PFGE patterns, with that of BP279 being most different from the rest, consistent with results of whole genome and pertactin sequence analysis. Together, these results indicate the presence of multiple *B pertussis* strains.

Vaccination Status

As shown in Table 1, 84.5% of patients with and 59.8% of patients without *B pertussis* had some documentation of childhood pertussis immunization, with 76.2% and 51.9% considered upto-date at presentation. Patients with *B pertussis* were less likely to have received whole-cell pertussis vaccine than those without *B pertussis* (8.1% vs 16.7%, respectively; *P*=.02), despite similar proportions having received the 2011-2012 influenza vaccine (54.4% vs 59.0%, respectively; *P*=.36).

Demographic Characteristics and Clinical Data

The age distribution of those with pertussis is shown in Supplemental Figure 1 (available online at http://www.mayoclinicproceedings.org). Patients who had pertussis were younger than those without pertussis (15.6 vs 25.5 years; *P*=.002). The mean time from symptom onset to Bordetella PCR testing was 13.1 and 16.4 days, respectively, in the 2 groups (*P*=.08). Patients with and without *B pertussis* had similar rates of hospitalization (3.8% vs 4.6%; *P*=.68) and previous respiratory illness (asthma or chronic obstructive pulmonary disease; 16.9% vs 15.5%; *P*=.71). Rates of post-tussive emesis were similar between those with positive and negative PCR findings (18.1% vs 13.8%; *P*=.24). No deaths were attributable to pertussis.

Time Since Last Vaccination in Patients Who Received Acellular Pertussis Vaccine Only

The time from the date of the most recent acellular pertussis vaccination to the date a patient underwent testing for *B pertussis* was examined among those who had received 5 or more doses of acellular pertussis vaccine (and no whole-cell vaccine). The mean (SD) time since the most recent pertussis vaccination in those with *B pertussis* (n=93) was 1363 days (818 days), compared with 1010 days (879 days) among those without *B pertussis* (n=51; *P*=.004).

B parapertussis Cases

Twenty-one patients had *B parapertussis*, all of whom had been age-appropriately vaccinated against *B pertussis*. Patients with *B parapertussis* were younger than those with

B pertussis (mean ages, 3.8 and 15.6 years, respectively; $P<.0001$). No hospitalizations or deaths were attributed to *B parapertussis*.

DISCUSSION

In 2012, southeastern Minnesota experienced its largest recent epidemic of pertussis. Our data support several possible explanations for the resurgence of pertussis our region, including genetic changes in the etiologic agent (*B pertussis*) associated with vaccine escape, waning of immunity conferred by acellular vaccines, and decreased efficacy of acellular compared with the whole-cell vaccines previously in use. The outbreak, which occurred in a geographically restricted area over a short time span, consisted of multiple circulating strains, consistent with findings in other pertussis outbreaks.[24]

All *B pertussis* isolates recovered from the outbreak (which represent a subset of patients studied) putatively lack expression of pertactin, an important component of acellular pertussis vaccines currently in use. Pertactin-negative *B pertussis* has been increasingly reported,[22,25-28] but to our knowledge, this is the first time that only pertactin-negative strains have been recovered during an outbreak. The finding of 9 isolates with the *prn1* allele with the 5' signal sequence deletion is unusual among *B pertussis* isolates from the United States.[23] The recovery of pertactin-negative strains in the setting of widespread vaccine utilization implies selective pressure and microbial adaptation. Because pertactin is a component of all currently used acellular vaccines, the presence of pertactin-negative *B pertussis* has potential implications for future vaccine design. Interestingly, pertactin-deficient *B pertussis* can outcompete pertactin-producing *B pertussis*, suggesting that pertactin deficiency may not negatively affect microbial fitness.[21]

The immunity conferred by any vaccine, including pertussis vaccine, wanes over time.[5,29] We found that among patients who had received at least 5 doses of acellular pertussis vaccine, the time since the last acellular vaccination was more than 350 days longer (on average) in those who had test results positive for *B pertussis* than in those who had negative results. These data suggest waning immunity from the currently used vaccines as a contributor to the outbreak.

Growing evidence supports the decreased efficacy of acellular compared with whole-cell pertussis vaccines, and there is evidence that receipt of whole-cell pertussis vaccines is associated with a lower risk of pertussis.[30,31] This evidence is supported by our finding that proportionally fewer patients with *B pertussis* had received whole-cell vaccine.

Beyond these explanations, other theories have been offered for the increase in pertussis in recent years, including increased clinical awareness and availability of more sensitive diagnostic tests.[5,32] Polymerase chain reaction testing is more rapid and has increased sensitivity compared with culture. These advantages have clinical relevance because, given the clinical course of pertussis, the microbial load may be low by the time a diagnosis of pertussis is entertained.[10] The increased sensitivity of PCR over culture is supported by our data. In our convenience sample, PCR detected more cases (N=24) than did culture (N=12) with only one case detected by culture and not PCR. The lower real-time PCR crossing point

of the culture-positive vs the culture-negative specimens suggests that the increased detection by PCR vs culture relates to the ability of the former to detect specimens containing low numbers of organisms. We do not believe that the use of this more sensitive test accounted for the outbreak, although it most certainly enabled its recognition. We have been using PCR routinely to diagnose pertussis since 1995. As shown in Figure 4, the increase in the number of PCR test results positive for *B pertussis* preceded the increase in clinical ordering of PCR testing, suggesting that increased utilization of PCR was not exclusively responsible for the increased number of cases diagnosed.

Interestingly, although there was an overall increase in the number of pertussis cases detected by PCR in 2012, infections in patients less than 1 year old declined while infections diagnosed by PCR increased among those aged 5 to 19 years when compared with national trends in the late 1990s. That there were no cases of pertussis in neonates may be due to recent changes in recommendations for the immunization of pregnant women. In 2011, the Advisory Committee on Immunization Practices of the CDC recommended that pregnant women who were not yet vaccinated with the tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine receive it in the late part of the second trimester or the third trimester of their pregnancy or in the early postpartum period.[33] Since the initial recommendation, repeated Tdap vaccinations with each subsequent pregnancy have been advocated by the Advisory Committee on Immunization Practices.[34] The lower number of pertussis cases in infants in our study than reported in previous eras[1] (Supplemental Figure 2, available online at http://www.mayoclinicproceedings.org) may be due at least in part to changes in vaccination protocols for pregnant women. This finding may be helpful because there is little available data supporting the efficacy of the new Advisory Committee on Immunization Practices recommendation.[35]

The PCR assay used in our laboratory detects both *B pertussis* and *B parapertussis*. Interestingly, 21 cases of *B parapertussis* infection were detected by PCR in 2012. Pertussis vaccines are unlikely to provide protection against *B parapertussis*, which can cause illness similar to that caused by *B pertussis*.[36] Consistent with lack of protection from the vaccine, our patients infected with *B parapertussis* were younger than those infected with *B pertussis* ($P<.001$), and all patients with *B parapertussis* had been appropriately vaccinated for pertussis. The mean age of patients with *B parapertussis* was 3.8 years, which is similar to that recently reported by Spicer et al[37] in Ohio (4.2 years). The reason(s) for the cocirculation of *B pertussis* and *B parapertussis* is unclear, but studies in a rodent model have suggested that acellular pertussis vaccination may impair clearance of *B parapertussis*.[38]

There are multiple limitations to our study. The vaccination history was incomplete for some patients, especially those with results negative for *B pertussis* and *B parapertussis*. These patients tended to be older and were therefore more likely to have been born during the whole-cell vaccine era. The lack of childhood immunization data for a larger proportion of those with negative than with positive results for *B pertussis* may have led to a falsely lower documented rate of vaccination with the whole-cell vaccine in those with negative results. Had complete records been available, it is likely we would have detected an even larger difference between the groups with respect to the proportion vaccinated with whole-cell

vaccine. A second limitation is that culture of nasopharyngeal swabs was performed using Regan-Lowe medium and there was variable time between specimen collection and culture. Enhanced culture techniques (eg, cough plates, bedside plating, Bordet-Gengou medium) may have improved the sensitivity of culture. A final limitation is the use of a PCR assay for *B pertussis* targeting the IS481 insertion sequence, which is also present in *B holmesii*. *B holmesii* was first characterized in the 1990s as a cause of bacteremia and sepsis and has subsequently been found to cause a respiratory illness similar to *B pertussis*.[1] IS481 is present in higher copy numbers in *B pertussis* (50-238 copies per genome) than in *B holmesii* (5-8 copies per genome).[39] Several studies have attempted to characterize the role of *B holmesii* in reported pertussis outbreaks and have come to varying conclusions. Estimates in smaller studies vary from 0.6% (Chile, 2010-2011) to 20% (France, 2009-2010) to 29% (Ohio, 2010-2011) of pertussislike illnesses.[37,39-41] However, a retrospective study of over 11,000 PCR tests of patients from Finland and the Netherlands with suspected pertussis (1992-2005) detected no *B holmesii*, despite finding 1856 PCR tests positive for pertussis using the *IS481* target.[42] We are unable to estimate what, if any, proportion of our cases were due to *B holmesii*; we were unable to isolate *B holmesii* because Regan-Lowe media contains cephalexin, which is inhibitory to *B holmesii*. However, given the above cited reports suggesting that *B holmesii* accounts for fewer than 30% of pertussis cases, the outbreak reported herein would still involve a greater number of cases than in previous years. Further, 12 of the *B pertussis* cases were confirmed by culture.

CONCLUSION

In 2012, our region experienced its largest local pertussis outbreak in recent history. The outbreak included multiple circulating strains, all of which putatively lack expression of pertactin, a target of the acellular pertussis vaccine. Fewer patients who had pertussis had received whole-cell pertussis vaccine, and the time since the most recent dose of acellular pertussis vaccine was longer in those patients with pertussis than in those without. Taken together, these data support decreased efficacy of acellular compared with whole-cell pertussis vaccine and waning immunity from acellular vaccines as possible contributors to this outbreak. We also found circumstantial evidence that recent increased vaccination efforts in pregnant women may have impacted neonatal pertussis.

Although the current vaccine may be suboptimal with respect to immunogenicity and durability of induced immunity, it is unlikely there will be a return to the use of the whole-cell vaccine given its association with adverse events and reactogenicity. In the absence of changes in vaccine strategy, we anticipate ongoing endemic pertussis activity with peaks in the late summer months and epidemics every 2 to 5 years, as occurred in the prevaccine era. Areas of the world where acellular vaccines were adopted later than in our area should anticipate the possibility of upcoming pertussis outbreaks. Acellular vaccines are safe; given possible success in the reduction in neonatal pertussis, decreases in pertussis incidence may be realized with increased frequency of acellular vaccine administration and/or deployment of improved pertussis vaccines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and Acronyms

CDC	Centers for Disease Control and Prevention
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
Tdap	tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis

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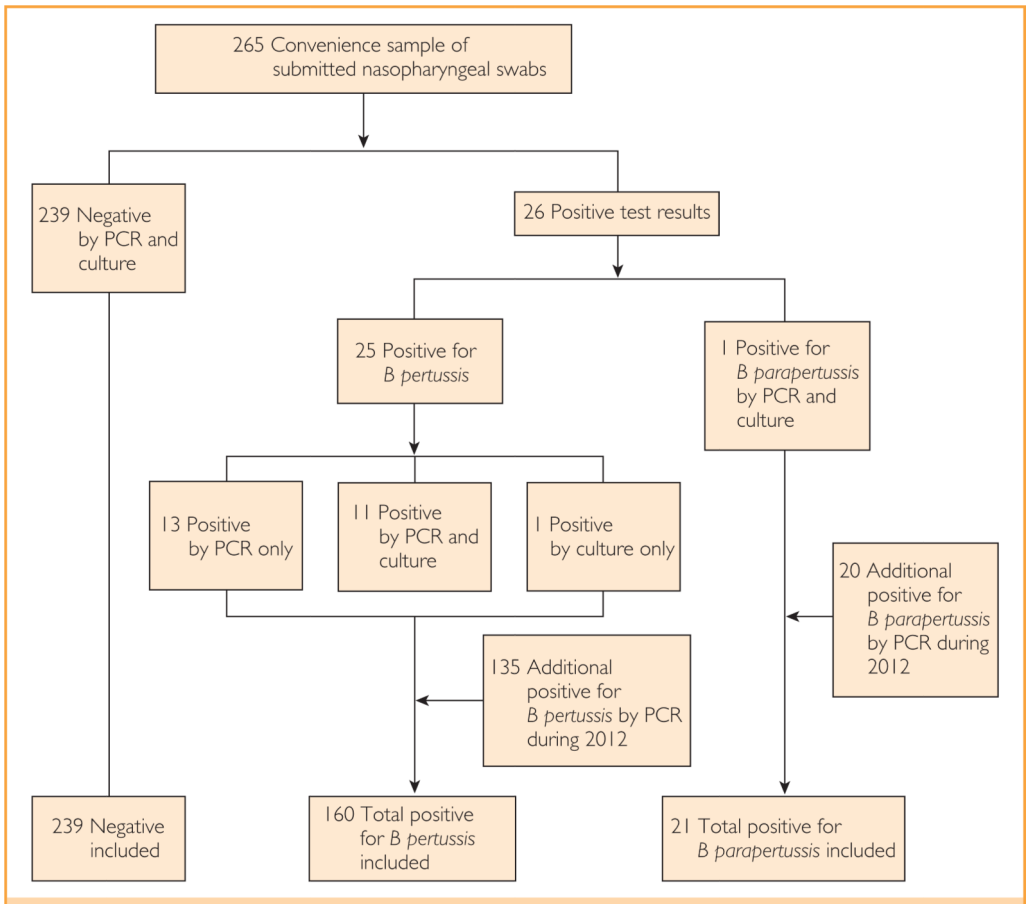

FIGURE 1.

Study flowchart. *B pertussis* = *Bordetella pertussis*; *B parapertussis Bordetella parapertussis*; PCR = polymerase chain reaction.

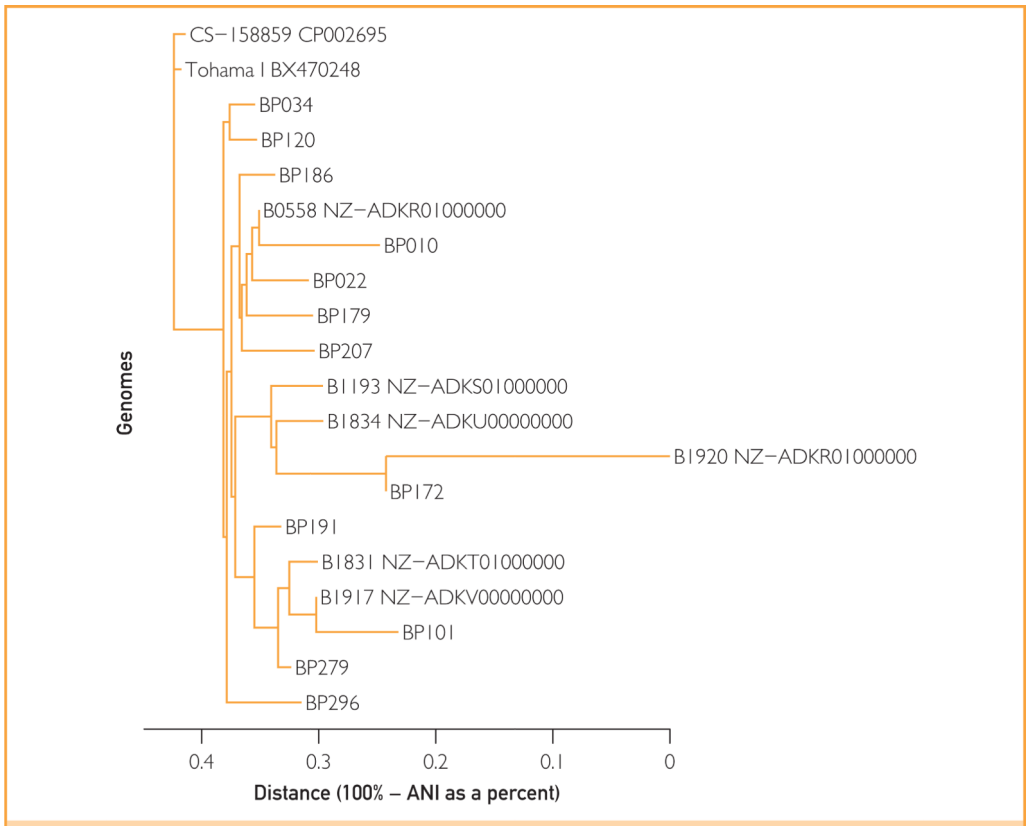

FIGURE 2.

Phylogenetic tree of genomic sequences of the 12 study *Bordetella pertussis* isolates compared with strains from GenBank (analysis based on average nucleotide identity [ANI]).

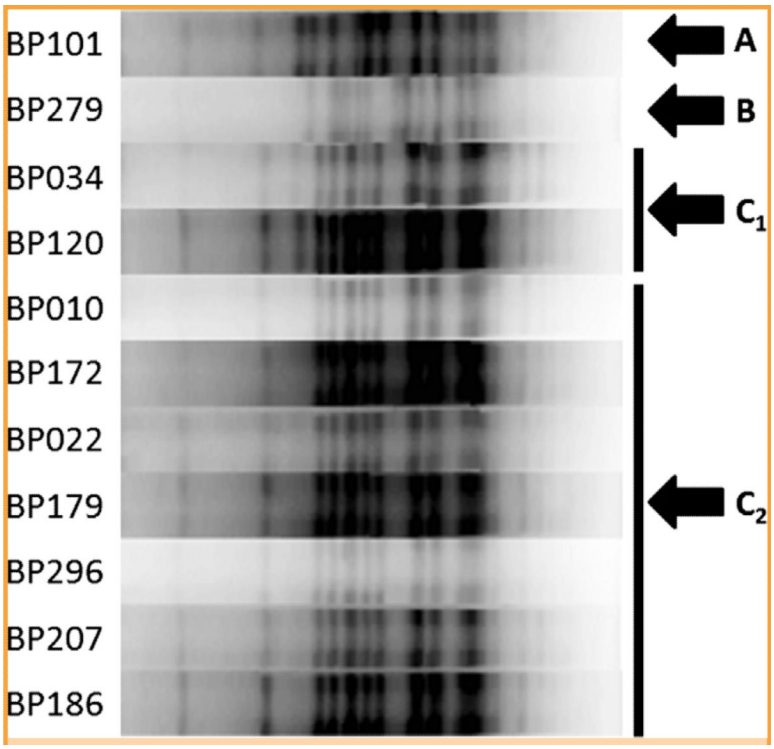

FIGURE 3.

Pulsed-field gel electrophoresis of 11 *Bordetella pertussis* isolates. Four patterns, A, B, C_1, and C_2, were observed, with B (associated with BP279) being the most different from the rest.

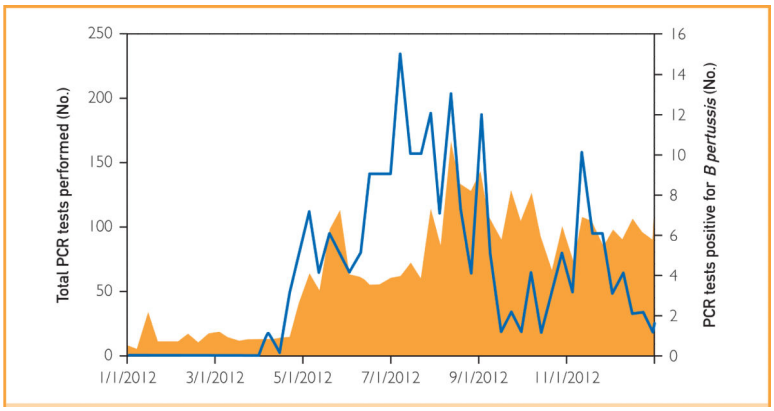

FIGURE 4.

Total number of polymerase chain reaction (PCR) tests for *Bordetella pertussis* and *Bordetella parapertussis* performed at Mayo Clinic in Rochester, Minnesota, in 2012 (orange area, left y-axis) and the number of PCR tests positive for *B pertussis* by month (blue line, right y-axis).

TABLE 1

Baseline Characteristics, Symptoms, and Vaccination Status of Patients Who Underwent Testing for *Bordetella pertussis* and *Bordetella parapertussh*[a,b]

Variable	Negative for *B pertussis* and B *parapertussis* (N=239)	*B pertussis* cases (N=160)	*B parapertussis* cases (N=21)
Demographic characteristics			
Female	142 (59.4)	75 (46.9)	12 (57.1)
Age at presentation (y), mean (SD; range)	25.5 (23.5; 8 d-90 y)	15.6 (17.7; 35 d-99 y)	3.8 (2.1; 6 mo-10y)
Time to testing (d), mean (SD; range)	16.4 (40.6; 1-560)	13.1 (9.1; 2-56)	15.4 (14.3; 1-56)
Presumed B *pertussis* or B *parapertussis* contact recorded	44 (18.4)	62 (38.8)	11 (52.4)
Physician-recorded symptoms			
Cough	232 (97.1)	159 (99.4)	21 (100)
Cough productive of sputum	63 (26.4)	42 (26.2)	5 (23.8)
Nasal symptoms	118 (49.4)	57 (35.6)	11 (52.4)
Sore throat	73 (30.5)	28 (17.5)	5 (23.8)
Fever	60 (25.1)	17 (10.6)	5 (23.8)
Posttussive emesis	33 (13.8)	29 (18.1)	5 (23.8)
Documented history of asthma or COPD	37 (15.5)	27 (16.9)	1 (4.8)
Tobacco smoke exposure	31 (13.0)	13 (8.1)	1 (4.8)
Azithromycin treatment	88 (36.8)	158 (98.8)	19 (90.5)
Hospitalization	11 (4.6)	6 (3.8)	0(0)
Vaccination history			
Childhood pertussis vaccination records available	143 (59.8)	135 (84.4)	21 (100)
Pertussis vaccine up-to-date	124 (51.9)	122 (76.2)	21 (100)
Received whole-cell pertussis vaccine	40 (16.7)	13 (8.1)	0 (0)
Received 2011-2012 season influenza vaccine	141 (59.0)	87 (54.4)	15 (71.4)

[a] COPD = chronic obstmctive pulmonary disease.

[b] Data are presented as No. (percentage) unless indicated othewise.

TABLE 2

Genotypic and Phenotypic Antimicrobial Susceptibility Characteristics of *Bordetella pertussis* Isolates[a]

Isolate	Pertactin allele	Pertactin sequence variation	NCBI GenBank database closest match	23S sequence variation	Erythromycin disk diffusion diameter (mm)	MIC (Etest) (μg/mL)
BP010	*prn1*	Signal sequence deletion	AB670735.1	Not present	57	0.016
BP022	*prn1*	Signal sequence deletion	AB670735.1	Not present	59	0.023
BP034	*prn1*	Signal sequence deletion	AB670735.1	Not present	52	0.023
BP296	*prn1*	Signal sequence deletion	AB670735.1	Not present	57	0.064
BP120	*prn1*	Signal sequence deletion	AB670735.1	Not present	57	0.023
BP172	*prn1*	Signal sequence deletion	AB670735.1	Not present	58	0.023
BP179	*prn1*	Signal sequence deletion	AB670735.1	Not present	56	0.016
BP186	*prn1*	Signal sequence deletion	AB670735.1	Not present	60	0.023
BP207	*prn1*	Signal sequence deletion	AB670735.1	Not present	64	0.016
BP191	*prn2*	S481 disruption	KC445197.1	Not present	60	0.016
BP279	*prn2*	S481 disruption plus nonsynonymous sequence variation[b]	KC445197.1	Not present	58	0.064
BP101	*prn2*	Stop codon	KC445199.1	Not present	59	0.016

[a] MIC = minimum inhibitory concentration; NCBI = National Center for Biotechnology Information.

[b] BP279 has a nonsynonymous sequence variation (C1577G) compared with KC445197.1.