Bordetella pertussis and Chronic Cough in Adults

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To evaluate Bordetella pertussis as a cause of persistent cough in adults, we examined 201 patients who had a cough for 2–12 weeks and no pulmonary disease. We obtained the following at presentation: medical history, chest radiograph, respiratory function measurement, nasopharyngeal aspirate for polymerase chain reaction (PCR), nasopharyngeal swab specimen for culture, and a blood sample (acute serum). Four weeks later a second blood sample (convalescent serum) was obtained. Control sera were obtained from 164 age-matched healthy blood donors with no history of cough during the previous 12 weeks. Four patients were B. pertussis culture-positive; 11 (including the culture-positive patients) were B. pertussis PCR-positive; and 33, including 10 of the 11 PCR-positive patients, had serological evidence of recent B. pertussis infection. Pertussis-positive and -negative patients could not be discriminated by a history of cough. We conclude that B. pertussis infection is a common cause of persistent cough in adults. This is of concern, because these patients may be B. pertussis reservoirs from which transmission may occur to infants, in whom the disease can be devastating.

Cough is a major reason for contact with general practitioners, accounting for nearly 30 million visits a year to primary care physicians in the United States [1]. Cough may be caused by several factors, including microorganisms [1]. Bordetella pertussis infection [2–6] and in adult family members of children with B. pertussis infection [7] has been demonstrated by serological tests. The diagnosis of B. pertussis is often based on high antibody titers in a single serum sample [2–5]; in such tests, sensitivity and specificity depend on the selected diagnostic cutoff values [3]. Further, other bacteria might induce a serological response of filamentous hemagglutinin antibodies mimicking B. pertussis infection [8, 9]. Direct detection of the organism would therefore improve the diagnosis of B. pertussis infection. Nasopharyngeal culture for B. pertussis is known to be insensitive, but with the introduction of highly sensitive PCR [10–13], it may be possible to detect the bacteria in nasopharyngeal secretions in a higher number of adults with persistent cough.

In this study, we used culture, PCR, and serology to investigate the frequency of the presence of B. pertussis in adult patients with prolonged cough.

Materials and Methods

Subjects and sampling. Adult patients (aged ≥16 years) referred to the Department of Pulmonary Medicine at Aarhus University Hospital (Aarhus, Denmark) for prolonged cough during the period of November 1995 to May 1997 were evaluated for the study. The inclusion criteria were coughing for a period of 2–12 weeks, a normal chest radiograph, normal spirometry test findings, and no chronic cardiopulmonary disease. Of 247 patients evaluated for inclusion in the study, 201 fulfilled the inclusion criteria: 69 males and 132 females, aged 16–77 years (mean 45.1 ± 14.1 years). Forty-six patients were excluded, of whom 15 had cough for >12 weeks, 19 had abnormal respiratory function test findings, 4 had pneumonia, 1 had sarcoidosis, 1 had cardiomegaly, and 6 refused to participate.

The control subjects were composed of 164 healthy blood donors, 101 males and 63 females aged 18–63 years (mean ± SD, 38.3 ± 10.4 years), who had no history of cough during the previous 12 weeks. Sera from the control population were obtained during the same period as the specimens from patients.

Each patient’s age and sex were noted and the medical history was obtained, including information about smoking habits, fever, use of antibiotics, and cough. Chest radiography (with anteroposterior and lateral projections) and a spirometry test were performed. The following samples were obtained at the first visit: a nasopharyngeal aspirate for B. pertussis PCR, a calcium alginate nasopharyngeal swab specimen for B. pertussis culture, and a blood sample for B. pertussis serology (acute serum).

Four weeks later a second blood sample was obtained for B. pertussis serology from 188 patients (convalescent serum). Thirteen
patients refused a second blood sampling. The nasopharyngeal aspirate and the sera were stored at $-80^\circ C$ until tested. Sera were analyzed in batches at completion of the study. A serum sample to assess the geometric mean titer of pertussis toxin IgG antibody was obtained from each of the participants in the control population.

**Culture of B. pertussis.** The nasopharyngeal swab specimens were immediately plated on *B. pertussis*-specific media [13] and incubated at $37^\circ C$ with high humidity for 7 days. Identification was based on colony form, oxidase-positivity, microscopy of gram-negative rods, and reaction with fluorescein-conjugated *B. pertussis* antibody (Bacto FA Bp; Difco Laboratories, Detroit).

**PCR analysis of B. pertussis.** For DNA extraction, 100 $\mu$L of nasopharyngeal aspirate was treated with 20 $\mu$L of dithiothreitol (60 mg/mL; D0632, Sigma, Diesenhofer, Germany) and 30 $\mu$L of proteinate K (10 mg/mL; 745723, Boehringer Mannheim, Mannheim, Germany) for 2 h at 56°C; subsequently, 200 $\mu$L of Chelex 100 (90 mg/mL; 143-2832, Bio-Rad, Richmond, CA) [14] was added, heated at 98°C for 15 min, and centrifuged at 20,000 g for 15 min. Twenty $\mu$L of a solution containing 100 mM TRIS and 10 mM EDTA (pH 7.4) was mixed with 180 $\mu$L of the supernatant. Five $\mu$L was used for PCR analysis.

The nested *B. pertussis* PCR system described by He et al. was used [11]. This system detects 2–3 bacteria per reaction tube and is 100% specific when tested against 130 bacterial strains [11]. In a simulated sample consisting of 100 $\mu$L of TE buffer (10 mM TRIS and 1 mM EDTA [pH 7.5]) and a known amount of *B. pertussis* DNA, the detection limit was 375 fg of *B. pertussis* DNA, equivalent to $\sim$70–80 genome copies (assuming a genome size of 3750 kbp) [15].

The PCR product was visualized by agarose gel electrophoresis in the presence of ethidium bromide. A positive test was defined by a 124-bp band [11]. For every 10 specimens, a positive and a negative control were run. To test for substances inhibitory to PCR, we ran a parallel quantitative PCR for every sample, with 4 $\mu$L of the patient sample and a known amount of extracted *Chlamydia trachomatis* DNA as the target DNA for the PCR. Cycling parameters were as described elsewhere [16].

**Determination of pertussis toxin antibody.** ELISA was adapted from the method of Meade et al. [17]. All plated volumes were 50 $\mu$L. Polystyrene microtiter plates (NUNC-IMMUNOSORB maxisorb; NUNC, Roskilde, Denmark code no. 439454) were coated with pertussis toxin (1 $\mu$g/mL in carbonate buffer; pH 9.6) overnight at 4°C. Test and reference sera were diluted 2-fold in the plate in dilution buffer (pH 7.2) consisting of PBS, 1% v/v Trition-X 100 (Serva 37240, Serva Electrophoresis, Heidelberg, Germany), and 1% bovine serum albumin and were incubated for 2 h at 20°C or overnight at 4°C. After 30 min the color development was stopped with 100 $\mu$L of 2-M HSO$_4$, and the absorption was read at 492 nm. The potency of each serum was calculated with a reference line method and on log-log transformed data. A serological diagnosis of *B. pertussis* was based either on a 4-fold increase in level of IgG antibody to pertussis toxin or a single pertussis toxin IgG antibody value 2 standard deviations above the geometric mean in the control group [2, 5]. Paired sera from an individual were analyzed on the same ELISA microtiter plate. An assay control serum was analyzed on each microtiter plate to monitor assay variability.

Throughout the study, we used United States reference pertussis antiserum (human), lot 3 (Laboratory of Reference Reagents, Food and Drug Administration, Washington, DC), diluted 1 : 1000 in dilution buffer. For detection of bound IgG antibody to pertussis toxin, we used peroxidase-conjugated goat antibody to human IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD; catalog no. 074-1002), diluted 1 : 10,000 in dilution buffer and incubated for 1 h at 20°C. O-phenylene diamine was used as color substrate.

**Statistics.** For data analysis we used the Statistical Package for the Social Sciences version 6.0 (SPSS, Chicago) [18]. For dichotomous variables, the $\chi^2$ test was used, and for continuous variables, Student’s $t$-test or the Mann-Whitney nonparametric test. For paired comparisons, Wilcoxon’s nonparametric test was used. The coefficient of variation was calculated as the standard deviation divided by the mean value of the assay control serum values, expressed as a percentage. A $P$ value below 0.05 was considered statistically significant.

**Results**

The 201 patients had had persistent cough for a mean of 6.5 weeks at enrollment. Four (2%) of these patients were *B. pertussis* culture–positive. Eleven (5.5%) of the nasopharyngeal aspirates were *B. pertussis* PCR–positive, including those from the 4 culture-positive patients (table 1). PCR inhibitory substances were not found in any sample.

The mean titer of IgG antibody to pertussis toxin in the patients positive for *B. pertussis* by PCR was significantly higher than that in the control population ($P<.0001$). The geometric mean plus 2 standard deviations for IgG antibody to pertussis toxin in the control population was 123 U/mL (specificity 97.5%). Thirty-three patients had a level of IgG antibody to pertussis toxin $>123$ U/mL; these 33 included all the culture-positive patients and 10 of the 11 *B. pertussis* PCR–positive patients. No patient had a 4-fold increase in titer of IgG antibody to pertussis toxin from the first to the second examination. The mean titer of IgG antibody to pertussis toxin in acute sera was significantly higher than that in convalescent sera ($P<.004$). *B. pertussis* seropositivity was independent of

**Table 1. Characteristics of patients positive for Bordetella pertussis by PCR.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, sex</th>
<th>Duration of cough, weeks</th>
<th>Culture</th>
<th>PT IgG*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30, M</td>
<td>6</td>
<td>–</td>
<td>52</td>
</tr>
<tr>
<td>2</td>
<td>26, F</td>
<td>12</td>
<td>+</td>
<td>242</td>
</tr>
<tr>
<td>3</td>
<td>24, M</td>
<td>6</td>
<td>–</td>
<td>442</td>
</tr>
<tr>
<td>4</td>
<td>34, F</td>
<td>4</td>
<td>+</td>
<td>165</td>
</tr>
<tr>
<td>5</td>
<td>43, M</td>
<td>12</td>
<td>+</td>
<td>431</td>
</tr>
<tr>
<td>6</td>
<td>21, F</td>
<td>8</td>
<td>–</td>
<td>196</td>
</tr>
<tr>
<td>7</td>
<td>26, F</td>
<td>4</td>
<td>–</td>
<td>282</td>
</tr>
<tr>
<td>8</td>
<td>34, F</td>
<td>4</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>51, F</td>
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<td>+</td>
<td>1465</td>
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<tr>
<td>10</td>
<td>37, M</td>
<td>4</td>
<td>–</td>
<td>300</td>
</tr>
<tr>
<td>11</td>
<td>16, F</td>
<td>4</td>
<td>–</td>
<td>572</td>
</tr>
</tbody>
</table>

NOTE. ND, not done; +, positive; –, negative.

* Titer of IgG antibody to pertussis toxin at first visit (acute) and at 4 weeks (convalescent).
age and sex. The interassay coefficient of variation for the pertussis toxin IgG ELISA was 11.7%.

One patient was *B. pertussis* PCR–positive but had no pertussis toxin antibody response (table 1). The patient was a 34-year-old woman who had had a dry, paroxysmal cough for 4 weeks at the time of examination. There was no previous history of cough. She was not able to recall whether she had been vaccinated against *B. pertussis*. Her sister had also been coughing. On the day the patient was examined, no other patients with *B. pertussis* infection were seen in the ward.

The male/female ratio or age distribution for the patients with serological evidence of *B. pertussis* infection was no different than for patients without *B. pertussis* infection; nor did we find any difference in the frequency of fever, antibiotic treatment, smoking habits, or history of cough (weeks of coughing, dry or productive coughing, day or night coughing, or paroxysmal cough with vomiting). In addition, when comparing PCR-verified *B. pertussis*–infected patients with *B. pertussis* PCR–negative patients, no difference in the above-mentioned parameters could be shown. Coughing family members were more frequently reported for patients who were *B. pertussis* PCR–positive and serologically positive than for patients in whom the presence of *B. pertussis* could not be demonstrated by either of these tests (P < .01).

**Discussion**

Physicians generally consider the prevalence of pertussis in adults to be low [19, 20], and a recent review article on chronic cough did not mention *B. pertussis* as a cause [21]. However, studies in Germany [10] and the United States [2–5] indicate that the prevalence of *B. pertussis* in adults with cough might not be negligible. The present study of pertussis in adults with persistent cough was carried out in a population in which patients with plausible etiologies of cough other than infection were not assessed.

Using direct detection by culture and *B. pertussis* PCR, the pertussis bacteria or their DNA could be detected in 2% and 5.5% of patients with persistent cough, respectively. The higher detection rate of *B. pertussis* by PCR than by culture was of the same order of magnitude as described by Schmidt-Schlaepfer et al. [22]. The *B. pertussis* isolation rate by culture might have been even higher if nasopharyngeal aspirates had been used for culture; a Swedish study has indicated that more isolates can be obtained from aspirates than from nasopharyngeal swab specimens [23].

At the first examination, subjects in our study had been coughing for an average of 6.5 weeks, and it is possible that more patients would have been positive by culture or PCR if they had been examined at an earlier stage of disease [8, 24]. In the German vaccine efficacy trial, in which family members were examined when they had been coughing for 1 week, eight of 21 patients examined by PCR were *B. pertussis* PCR–positive [10]. Two of our patients were still *B. pertussis* culture–positive and PCR–positive after 12 weeks of cough; however, we cannot exclude the possibility that these 2 patients had pertussis as a superinfection.

Serological evidence of *B. pertussis* infection was found in 16.4% of the patients, including 10 of the 11 *B. pertussis* PCR–positive patients. Our finding that coughing family members were more frequent among patients with serological evidence of *B. pertussis* than among patients without serological evidence of pertussis supports the view that patients with positive serologies really had infection with *B. pertussis*, which is highly contagious.

A study in the United States and 1 in Australia in which all the diagnoses of *B. pertussis* were based on 1 high antibody titer, and 2 United States studies in which most of the diagnoses of *B. pertussis* were based on 1 high antibody titer—though few diagnoses were based on a 4-fold titer rise—found prevalences of *B. pertussis* of 12%–26% among adult patients with chronic cough [2–5]. Deville et al., who analyzed pertussis toxin antibodies 6× yearly over a period of 5 years, found evidence of an average annual pertussis infection rate of 8% [25]. No information on history was included in that study.

Our study population had a mixed background of pertussis vaccination status, and many patients had had *B. pertussis* infection in childhood, which may give a rapid anamnestic immune response. Thus the increase in titer of IgG antibody to pertussis toxin may be missed when “acute” sera are taken at a late stage of disease [26], and this might explain why none of our paired sera showed a 4-fold increase in titer of IgG antibody to pertussis toxin. However, we observed a decrease in such antibody titers from the first to the second examination, similar to the findings of Simondon et al. [27].

No difference in history of cough was found between groups with and without evidence of *B. pertussis* infection, whether we compared test results from culture, PCR, or serology. This is in agreement with the findings of Wright et al. [2] and Mink et al. [3], who concluded that illnesses of patients with pertussis were similar to the illnesses of patients without pertussis. However, in previous studies the diagnosis of *B. pertussis* infection has for some patients been based on history alone [28, 29]. Our study and the studies of Wright et al. [2] and Mink et al. [3] indicate that the diagnosis of *B. pertussis* infection should not be based on history alone and that a diagnostic method is needed, at least for adults.

PCR analysis seems superior to culture, and serology may be superior to PCR in an advanced stage of the disease. However, if the diagnosis of *B. pertussis* infection is based on a test for pertussis toxin antibodies in a single serum sample, sensitivity and specificity of the test depend on the diagnostic cutoff value selected [3]. If the cutoff values of mean titers of IgG antibody to pertussis toxin are set at plus 2 standard deviations, 2.5% of patients without pertussis would meet the case definition by statistical chance; if set at 3 standard deviations, 0.5%
of patients. Furthermore, patients with an antibody value near the diagnostic cutoff value might be misdiagnosed because of assay variability. Therefore, serology and PCR might be supplementary in the diagnosis of *B. pertussis* infection.

One patient was *B. pertussis* PCR–positive but failed to generate a pertussis toxin antibody response, which has been described in children with *B. pertussis* infection [8]. A false *B. pertussis* PCR result due to contamination might be possible, but our PCR laboratory is organized according to the guidelines of Kwok and Higuchi [30], and the negative controls were all negative during the study.

In conclusion, we demonstrated the presence of *B. pertussis* by culture or PCR analysis in 5.5% of adult patients who had persistent cough for 2–12 weeks. If patients had been examined at an earlier stage of disease, the frequency of *B. pertussis* culture–positivity and PCR-positivity might have been even higher, because 16.4% of the patients had serological evidence of a recent *B. pertussis* infection.

Detection of the microorganism by culture or PCR in symptomatic patients implies that the patient is or has been highly contagious. Physicians should be aware of the impact of *B. pertussis* on coughing in adults, regardless of history of cough, because these patients may be a reservoir for *B. pertussis* and could potentially transmit the infection to infants, in whom the disease can be devastating.

Acknowledgments

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References