**Bordetella parapertussis** Infection in Children: Epidemiology, Clinical Symptoms, and Molecular Characteristics of Isolates

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The clinical trial conducted in Italy to evaluate the efficacy of acellular pertussis vaccines provided an opportunity to estimate the frequency of clinical infections with *Bordetella parapertussis* and to compare the clinical characteristics of children suffering from *Bordetella pertussis* illness with those of children with *B. parapertussis* illness. This study dealt with 76 *B. parapertussis* infections diagnosed from a population of 15,601 children participating in the follow-up of suspected cases of pertussis. An overall incidence of 2.1 cases of laboratory-confirmed parapertussis per 1,000 person-years was observed. Children affected by *B. parapertussis* infections showed a less severe clinical picture both in the duration of symptoms and in the percentage of patients affected, even when compared with vaccinated children with *pertussis*. To characterize the isolated strains, we performed assays for susceptibility to erythromycin and sulfamethoxazole-trimethoprim, and we examined the genomic DNAs by pulsed-field gel electrophoresis. The results showed a high degree of genetic stability among *B. parapertussis* strains regardless of time of collection and geographical distribution.

Whooping cough is mostly associated with *Bordetella pertussis* infection, but *Bordetella parapertussis* is also responsible for a whooping cough-like disease (14, 16, 21). The latter generally has a milder clinical presentation (10), but it is not easily distinguished from *B. pertussis* infection by symptoms, and usually it is not laboratory confirmed. For these reasons the epidemiology of illness caused by *B. parapertussis* is poorly recognized.

*B. pertussis* and *B. parapertussis* share a number of virulence factors, such as filamentous hemagglutinin (FHA), pertactin, tracheal cytotoxin, dermonecrotic toxin, and adenylate cyclase-hemolysin (2, 4, 6, 15, 18). However, the pertussis toxin (PT) gene is not transcriptionally active within the promoter and the coding regions (1). In spite of the similarities, differences in protective epitopes for common antigens and the nonexpression of PT in *B. parapertussis* may explain the lack of cross-protection between the two species.

During the recent randomized, placebo-controlled clinical trial conducted in Italy to evaluate the efficacy of new acellular pertussis vaccines (7), we were also able to estimate the incidence of *B. parapertussis* infections and to compare the clinical pictures of *B. parapertussis* and *B. pertussis* infections.

Furthermore, in order to characterize the isolates of *B. parapertussis* circulating in our country, we performed susceptibility testing on *B. parapertussis* isolates and examined the DNA by macrorestriction digestion. Recent studies have demonstrated that the analysis of DNA fragments, generated by rare-cutting restriction enzymes in pulsed-field gel electrophoresis (PFGE), can be profitably applied to detect the clonal origin and genetic relatedness for *Bordetella* spp. (11, 22).

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**MATERIALS AND METHODS**

**Patients, sampling, and clinical data collection.** A total of 15,601 infants at the age of 2 months were enrolled in the trial between September 1992 and September 1993 and were randomly assigned to four vaccine groups (7). Thirty percent of the infants received a diphtheria-tetanus-whole cell pertussis vaccine (DTPw) manufactured by Connaught Laboratories (Swiftwater, Pa.), 30% received a diphtheria-tetanus-acellular pertussis vaccine (DTaP CB) manufactured by Chiron Biocine (Siena, Italy), 30% received a diphtheria-tetanus-acellular pertussis vaccine (DTaP SB) manufactured by SmithKline Beecham Biologicals (Rixensart, Belgium), and 10% received a diphtheria-tetanus vaccine (DT) manufactured by Chiron Biocine.

Active surveillance of coughing was implemented for all children. Nasopharyngeal aspirates (NPAs) and paired serum samples were collected from children with coughs lasting more than 7 days.

Parents of children with coughs recorded symptoms daily in a standardized diary; a trained nurse contacted the family each week to review and record this information.

The results of the trial (7) indicated that both acellular pertussis vaccines (DTaP CB and DTaP SB) had high clinical efficacy (84%) against pertussis, whereas DTPw showed poor efficacy (36%). In this study the clinical symptoms of 773 children with *B. pertussis* infections were compared with those of 76 children with *B. parapertussis* infections diagnosed during the period September 1992 to September 1995. Sixty-seven *B. parapertussis* strains isolated from 76 patients with infections were assayed for phenotypic and molecular characteristics.

**Laboratory procedures.** (i) **Culture.** For primary isolation from NPAs, bacteria were grown on charcoal agar plates supplemented with cephalxin (20 μg/ml) (Unipath, Milan, Italy) and incubated at 35°C in a moist atmosphere for 7 days. All suspected colonies were identified by biochemical tests (oxidase, urease) and by agglutination with antisera specific for *B. pertussis* and *B. parapertussis* (Murex Diagnostics, Dartford, England) and were confirmed by PCR (23). *B. parapertussis ATCC 9305* and *B. pertussis ATCC 9779* were used as controls.

(ii) **Serology.** Paired capillary blood samples were collected in the acute and convalescent phases from children with coughing episodes. Geometric mean titers of antibodies to PT and FHA were measured by a standardized enzyme-linked immunosassay (17). As in other studies (7, 8, 19, 24), an increase to at least twice the initial value in the level of immunoglobulin G (IgG) or IgA antibody to either PT or FHA was considered significant for the diagnosis of pertussis, provided the intra-assay coefficient of variation was less than 20%.

To discriminate between *B. pertussis* and *B. parapertussis* infections when the serological test result was positive only for the common FHA antigen and the relative asparagine was culture negative, a PCR assay for the detection of *B. parapertussis* DNA in the aspirate was performed by using experimental parameters already described (23, 27).

(iii) **Antimicrobial susceptibility testing.** Assays for susceptibility to erythromycin and sulfamethoxazole-trimethoprim were performed on *B. parapertussis* isolates by using the E-test method (AB Biodisk, Solna, Sweden) according to the manufacturer's package insert.

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RESULTS

Clinical symptoms. At the end of September 1995, 849 Bordetella sp. infections had been diagnosed, 544 by culture and 305 by serology. In particular, 76 B. pertussis infections occurred in males, and the mean age of these children was 15.4 months (range, 2 to 36 months). The two types of infection showed similar seasonal trends, with an increase in cases from April to July, as shown in Fig. 1. The frequencies of symptoms in children with B. parapertussis and B. pertussis infections who had been vaccinated with the DT vaccine or one of the pertussis vaccines are shown in Table 1.

Children with B. parapertussis infections had a significantly lower frequency of all symptoms than all groups of children with B. pertussis infections except the DTaP CB vaccines. Figure 2 shows the mean duration of symptoms by group. In particular, a significantly shorter duration of coughing (mean duration, 25 days) than all groups of children vaccinated with pertussis vaccines after completion of three doses of vaccine. In particular, we observed an incidence of 0.7 cases of parapertussis infections per 1,000 person-years in unvaccinated children (4 cases) compared to incidences of 1.3 in children vaccinated with DTPw (23 cases), 1.0 in DTaP CB recipients (22 cases), and 1.9 in the DTaP SB group (27 cases).

Antimicrobial susceptibility testing. All B. parapertussis strains were susceptible to erythromycin, the antibiotic of choice for treating whooping cough disease: the MIC at which 90% of the isolates were inhibited (MIC for) was 0.5 μg/ml. Only 2 of the 67 B. parapertussis strains were moderately susceptible to erythromycin, with MICs for the strains being 2 and 3 μg/ml. All the serological results, the duration of coughing was 13 to 41 days (mean, 23 days), while those with positive serological results experienced a duration of coughing from 11 to 53 days (mean, 20 days).

An overall incidence of 2.1 B. parapertussis infections per 1,000 person-years was observed. Of 76 B. parapertussis infections, 51% occurred in males, and the mean age of these children was 15.4 months (range, 2 to 36 months). The two types of infection showed similar seasonal trends, with an increase in cases from April to July, as shown in Fig. 1. The frequencies of symptoms in children with B. parapertussis and B. pertussis infections who had been vaccinated with the DT vaccine or one of the pertussis vaccines are shown in Table 1.

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![Diagram](http://jcm.asm.org/)

TABLE 1. Frequencies of symptoms of children with B. parapertussis and B. pertussis infections, by vaccine group

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. (%) of B. parapertussis patients (n = 76)</th>
<th>No. (%) of B. pertussis patients (n = 773) vaccinated with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>76 (100)</td>
<td>130 (100) 156 (100) 343 (100) 144 (100)</td>
</tr>
<tr>
<td>Paroxysm</td>
<td>58 (76)</td>
<td>108 (83) 137 (88) 317 (92) 138 (96)</td>
</tr>
<tr>
<td>Whooping</td>
<td>25 (33)</td>
<td>60 (46) 77 (49) 233 (68) 116 (81)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>32 (42)</td>
<td>73 (56) 97 (62) 275 (80) 120 (83)</td>
</tr>
<tr>
<td>Apnea</td>
<td>22 (29)</td>
<td>47 (36) 77 (49) 226 (66) 117 (81)</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>9 (12)</td>
<td>29 (22) 45 (29) 159 (46) 88 (61)</td>
</tr>
</tbody>
</table>
isolates were also susceptible to sulfamethoxazole-trimethoprim; the MIC<sub>90</sub> was 0.125 μg/ml.

PFGE. Macrogenomic fingerprinting of the DNAs of the 67 <i>B. parapertussis</i> strains digested with <i>Xba</i>I produced 10 large fragments in the size range from 100 to 500 kb and numerous fragments smaller than 98 kb. These last fragments were not resolved adequately to provide useful information. The comparison of DNA patterns among the strains was based on the variation in the 10 largest fragments.

Two macrorestriction patterns were identified among the 67 isolates, and precisely 57 (85%) had a profile indistinguishable from that of <i>B. parapertussis</i> ATCC 9305 (pattern A), while 10 (15%) had a slightly different profile (pattern A1) characterized by a fragment of 250 kb instead of the 262-kb fragment present in pattern-A strains (Fig. 3).

The same isolates, when examined with the restriction enzymes <i>Spe</i>I and <i>Dra</i>I, showed identical patterns which could not be distinguished from that of the reference strain.

The CS calculated between patterns A and A1 was 0.9. No correlation was found between patterns and duration of coughing or severity of illness (paroxysms, etc.).

The circulation of pattern A1 in the regions participating in the clinical trial seemed to be limited to the northern regions, since it was not present in the southern region of Puglia.

**DISCUSSION**

The great interest that for years has been devoted to the whooping cough disease caused by <i>B. pertussis</i> has led to less attention to the closely related species <i>B. parapertussis</i>. The latter is also responsible for outbreaks of whooping cough-like disease in children, although it has been shown that clinical symptoms are generally milder (10).

In spite of the high degree of homology shown by the amino acid sequences of the main antigens, the two species differ with respect to several protective epitopes. In fact, antipertussis vaccines in an animal model (12) and even <i>B. pertussis</i> infections (25) do not seem to protect against <i>B. parapertussis</i> infections.

Assuming that pertussis vaccines are not efficacious in preventing <i>B. parapertussis</i> infections, as suggested also by this study, the incidence of <i>B. parapertussis</i> infection in Italian children 36 months of age or younger is 2.1 per 1,000 person-years. This figure does not, however, include asymptomatic cases or those with coughing lasting fewer than 7 days. Moreover, given the significant clinical picture observed, all the cases studied...
may be considered infections, even those in which serological responses were negative.

The analysis of clinical characteristics for children with *B. parapertussis* infections shows that the cough is accompanied by paroxysms in 76% of the cases and by posttussive vomiting in nearly 40%. However, the duration of the symptoms is significantly shorter for *B. parapertussis* infections than for *B. pertussis* infections in both vaccinated and unvaccinated children.

The similarity of symptoms observed in the comparison between *B. parapertussis* infections in all patients and *B. pertussis* infections in DTaP CB vaccinees points out the potential for clinical misdiagnosis in children vaccinated against pertussis. In this respect, PCR may be a valuable tool for differentiating *B. parapertussis* from *B. pertussis* in vaccinated and unvaccinated children.

In the future, a more accurate knowledge of the epidemiology of *B. parapertussis* will be necessary to define the potential development of an appropriate prophylaxis, which antipertussis vaccines do not seem to provide.

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REFERENCES


10. Heiningen, U., S. Klemens, S. Schmitt-Grohé, C. Lorenz, R. Rost, P. Chris-
tensen, M. Uberall, and J. D. Cherry. 1994. Clinical characteristics of *Bor-


tussis* infection followed by pertussis infection. *Lancet* 344:1703.


27. van der Zee, A., C. Agterberg, M. van Aertegeld, M. Peeters, and F. R. Mooi. 1993. Characterization of IS1001, an insertion sequence element of *Borde-