Pertussis in young infants: apnoea and intra-familial infection

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ABSTRACT

This study investigated 41 infants, aged <4 months, who were hospitalised with symptoms compatible with pertussis. Of these, 16 had *Bordetella pertussis* infection confirmed by real-time PCR. For four of these 16 patients, the initial sample was PCR-negative, but samples collected 5–7 days after the onset of infection were PCR-positive. PCR was also positive with samples from 15/16 families and 20/41 household contacts. Nine of the 20 positive household contacts were asymptomatic. Among the 16 infants with proven pertussis, apnoea was more frequent than in a control group for whom PCR was negative with both children and household contacts (69% vs. 28%). It was concluded that real-time PCR performed with samples from household contacts facilitates the diagnosis of infants suspected clinically of having pertussis, thereby enabling earlier treatment.

Keywords Apnoea, *Bordetella pertussis*, household contacts, infants, pertussis, real-time PCR

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INTRODUCTION

Pertussis is under-diagnosed in adults vaccinated during infancy in countries where a high vaccine coverage of infants and children has been achieved [1,2]. In France, children are first vaccinated at an age of 2 or 3 months, according to the vaccination recommendations [3]. A previous study conducted in the Paris area, which is a region with a vaccination coverage of >90% in infancy, showed that 30% of adult patients with a cough lasting >8 days and without an evident diagnosis had biological signs of *Bordetella pertussis* infection [4]. Atypical pertussis in adults contributes to the spread of the disease among non-immunised infants. The French National Pertussis network demonstrated that pertussis is diagnosed mainly in children aged <1 year (80% of cases), which is the age at which the disease can be most serious and life-threatening in conjunction with apnoea [2,5–7]. In France, pertussis is the most frequent cause of mortality associated with community-acquired bacterial infection in children aged <2 months [8].

The present study used a *Bordetella*-specific PCR to systematically screen the household contacts of young infants hospitalised with apnoea or paroxysmal or vomiting cough during the winter of 2004–2005, which was a season with known reported cases of pertussis in Paris. The aim of the study was to investigate the utility of such a systematic screen of household contacts in diagnosing the disease in children.

PATIENTS AND METHODS

A *B. pertussis*-specific real-time PCR (see below) was performed systematically on nasopharyngeal aspirates from all infants aged <4 months who were hospitalised with apnoea, with or without a cough, or for a paroxysmal or a vomiting cough, between 15 October 2004 and 15 March 2005. Apnoea occurred just before admission, while the onset of cough was 2–5 days previously. Samples from infants were collected upon admission, and were also collected systematically from household contacts, i.e., parents, siblings and grandparents, if they lived in the same dwelling. Household contacts were questioned concerning the presence, duration and nature of any cough (particularly during nocturnal sleep). Two groups of infants were then investigated: (i) infants with a positive diagnosis of pertussis (a positive PCR sample from the infant or from one household contact); and (ii) infants with a negative diagnosis of pertussis (PCR-negative samples from both
children and household contacts). The children were hospitalised in the general paediatric ward of Saint-Vincent de Paul Hospital, which is a teaching hospital situated in the centre of Paris. When the Bordetella-specific PCR was negative or uninterpretable with samples from an infant, but positive with samples from a household contact, the PCR was repeated after 5–7 days with samples from the infant.

Nasopharyngeal aspirates were obtained from young children, while a nasopharyngeal sample was collected with a Dacron swab from adults. Nucleic acids were extracted from clinical specimens with a QiaAmp DNA blood minikit (Qiagen, Hilden, Germany). All samples (200 μL) were extracted according to the manufacturer’s instructions. Real-time PCR was performed as described previously using Hotstar Taq Master Mix (Qiagen) [9] and primers specific for a 181-bp region of IS481 (accession no. L26973). The PCR profile comprised 10 min at 95°C, followed by 50 cycles of 10 s at 95°C, 10 s at 55°C and 20 s at 72°C. Amplification, detection and data analysis were performed with a Light Cycler 2.0 (Roche Diagnostics, Meylan, France). Negative controls with no template were included in each PCR run and after every ten samples.

All infants were also screened systematically for respiratory syncytial virus (RSV), influenza virus, parainfluenza virus and adenovirus using standard virological diagnostic procedures.

RESULTS

Bordetella-specific PCR was performed with samples from 41 infants aged <4 months and with samples from 80 household contacts. The diagnosis of pertussis was confirmed by PCR (group 1) for 16 infants (three patients aged <1 month, five aged 1–2 months, and eight aged 2–4 months). For 12 infants, the first PCR was positive; for four other infants, the initial PCR was negative, but was positive for one of the household contacts. A second PCR, performed with samples collected after 5–7 days, was positive for three of these four patients. The remaining patient, a boy aged 3 months, yielded a negative second PCR result, but had typical whooping cough with paroxysms, and the mother’s PCR result was positive. Overall, for four of the 16 patients, a positive Bordetella-specific PCR with samples from the parents helped to confirm the diagnosis of pertussis infection in the infants.

Of these 16 infants, 11 had apnoea, alone in three cases and associated with cough in eight cases. Five other infants had a paroxysmal cough with vomiting, with three being hospitalised for RSV bronchiolitis. Among the 11 infants with apnoea, eight were admitted to the paediatric intensive care unit (PICU). Overall, six of 16 infants were co-infected with RSV. No other viral infections were detected.

In 15 of 16 families of the positive infants, at least one household contact had a positive Bordetella-specific PCR result. In one family, only the mother was tested and she was negative. Overall, 20 (48.8%) household contacts yielded a positive Bordetella-specific PCR result. The positive household contacts comprised nine (56.2%) of 16 mothers, four of ten fathers (six were not tested), five (45.4%) of 11 siblings, and one of four grandparents. In seven of the 16 cases, the infant was the first child and was living with his parents only. Of the 20 household contacts positive for B. pertussis, only ten had a cough lasting for >5 days, with waking during nocturnal sleep in four cases. Among the other household contacts, four did not cough, and five had a mild cough lasting <5 days, which was considered to be common during the winter season (Table 1).

All the parents and siblings living with the infants had received a whole-cell pertussis vaccine during childhood. This information was not available for the four grandparents living in the household. No sibling aged <6 years was PCR-positive, and two brothers (aged 7 and 8 years) who were PCR-positive did not have a cough.

Patients and household contacts positive for B. pertussis received treatment with macrolides (clarithromycin or azithromycin). In the infants, apnoeas eased within 2–4 days of commencing treatment, but the cough took longer to disappear. Two of these 16 infants had received a single dose of acellular pertussis vaccine. The others had not received any vaccine.

During the same period, 26 other infants aged <4 months were hospitalised with symptoms resembling pertussis, i.e., apnoea in seven (28%) cases (alone in two cases and apnoea with cough in five cases), or vomiting cough (18 cases), with four of these 18 cases having a cough with paroxysms on the day of admission. All of these infants were negative when tested using the Bordetella-specific real-time PCR (group 2). The real-time PCR was also performed with specimens from 39 household contacts, including 23 mothers and 16 fathers, but was negative on every occasion. The apnoea and/or cough were probably related in most cases to gastro-oesophageal reflux or viral infection; indeed, 13 infants were infected with RSV. Five of these 26 infants were admitted to the PICU. The four infants who had paroxysmal cough were all...
positive for RSV, and the paroxysms stopped 24–48 h after admission.

Overall, during the winter season investigated, six infants aged <4 months with RSV-positive bronchiolitis were admitted to the PICU. Of these, three had a positive *Bordetella*-specific PCR result in association with the viral infection. No other viral infections were detected.

**DISCUSSION**

Pertussis is under-diagnosed in countries with high vaccine coverage, often because the symptoms are atypical, especially in teenagers and adults vaccinated during their childhood [10,11]. Severe morbidity and mortality are greatest among infants, particularly those who are either not immunised or not completely immunised. In the USA, it has been estimated that >70% of deaths caused by pertussis were not properly reported in 1994–1995 [6]. Teenagers and adults vaccinated during childhood contribute to the spread of the disease, and are a reservoir of infection for very young infants, in whom pertussis may be severe and life-threatening [1,2,10,11]. Recommendations for booster immunisations of teenagers in the USA [10,11] and in young parents (before the birth for fathers, and just after the birth for mothers) in France [3] are not generally followed.

Diagnosis of pertussis relies mainly on clinical symptoms, as bacterial cultures are often negative. Serological testing can help to diagnose atypical pertussis, but does not provide timely results because of the necessity to compare two successive sera [1]. Real-time PCR for *Bordetella* allows a quick diagnosis, but may be unreliable because of low bacterial numbers in the respiratory tract, particularly at the beginning of the disease. In the present study, the *Bordetella*-specific PCR was initially negative or uninterpretable in four of 16 cases, but was positive with a second specimen in three cases. The infants were in the first phase of the disease, and only five of 16 had whooping cough with paroxysms; however, the same symptoms were also present upon admission for some infants with RSV-positive bronchiolitis who yielded a negative *Bordetella*-specific PCR.

The present study demonstrated that testing of household contacts is useful in confirming a diagnosis of pertussis in infants, even if the parents do not have specific symptoms. Indeed, among the 20 household contacts of the children positive for *B. pertussis*, only one had the typical symptoms of pertussis, although ten had been coughing for >5 days. The remainder had been coughing for <5 days, or not at all in four cases. These results suggest that transmission of *B. pertussis* can occur via asymptomatic adult carriers in close contact with incompletely vaccinated young infants. Of the 16 children with pertussis, 14 were non-vaccinated and two had received only one dose, which is not sufficient to protect against pertussis.

The 41 infants tested for pertussis had identical symptoms, but the 16 infants with a positive *Bordetella*-specific PCR presented more often with apnoea (69% vs. 28%). These observations should be confirmed in larger studies. The 25 infants with a negative *Bordetella*-specific PCR could not be differentiated clinically from the 16 positive cases upon admission, but the household contacts of these 25 infants were all PCR-negative. These results suggest that a *Bordetella*-specific PCR should be performed systematically on household contacts following admission of an infant, even in the absence of specific symptoms, to help to confirm a possible diagnosis of pertussis in the infant. It is important to note that viral infections do not exclude an association with pertussis; thus, three of six infants presenting with RSV-positive bronchiolitis and admitted to the PICU had an associated pertussis infection [7].

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**Table 1.** Results of real-time *Bordetella*-specific PCR for 41 infants and their household contacts

<table>
<thead>
<tr>
<th></th>
<th>Group 1 Pertussis-positive</th>
<th>Group 2 Pertussis-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>15 1*</td>
<td>0/25</td>
</tr>
<tr>
<td>Index case (n = 41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apnoea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11 (66.7%)b</td>
<td>7 (28%)</td>
</tr>
<tr>
<td>Apnoea alone</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Apnoea + cough</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Paroxysmal or vomiting cough</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>RSV-positive</td>
<td>6 (37%)</td>
<td>13 (52%)</td>
</tr>
<tr>
<td>Household contacts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household members</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20/41 (48.8%)</td>
<td>0/39</td>
</tr>
<tr>
<td>Mothers</td>
<td>9/16 (56.2%)</td>
<td>0/23</td>
</tr>
<tr>
<td>Fathers</td>
<td>4/10 (25%)</td>
<td>0/16</td>
</tr>
<tr>
<td>Siblings</td>
<td>5/11 (45.4%)</td>
<td></td>
</tr>
<tr>
<td>Grandparents</td>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td>No cough (or cough &lt;5 days)</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>Cough ≥5 days</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Paroxysmal or vomiting cough</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*Typical whooping cough with PCR-negative result, but PCR-positive household contact.

bp <0.01.

R SV, respiratory syncytial virus.

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It is accepted widely that early antibiotic treatment reduces clinical symptoms, and consequently may reduce the mortality and the length of hospitalisation [2]. In young infants with a pertussis infection at its early stage, antibiotic treatment can diminish the severity of symptoms and the complications [12]. In the USA, 56 of 62 children who died of pertussis infection at the end of the 1990s were aged <6 months [2,6], and a recent French study showed that pertussis was the most important cause of mortality associated with community-acquired bacterial infection before the age of 2 months [8]. Therefore, diagnosis should be made as quickly as possible in young infants, especially in cases of apnoea, to enable the initiation of antibiotic treatment. Testing household contacts may allow the detection of intrafamilial infection, leading to an earlier diagnosis of pertussis in young infants, and making the additional expense of PCR cost-effective [7].

REFERENCES