**Significant Finding of Bordetella holmesii DNA in Nasopharyngeal Samples from French Patients with Suspected Pertussis**

Elisabeth Njamkepo,1,2 Stéphane Bonacorsi,3 Monique Debruyne,4 Sophie Anne Gibaud,5 Sophie Guillot,1,2 and Nicole Guiso1,2*

Institut Pasteur, Molecular Prevention and Therapy of Human Diseases, National Centre of Reference of Whooping Cough and Other Bordetelloses,1 and CNRS-URA 3012,2 Paris, France; Hôpital Robert Debré, Service de Microbiologie, Paris, France3; Laboratoire Cerba, Saint Ouen L'Aumône, France4; and Laboratoire de Bactériologie, Centre Hospitalier Universitaire de Nantes, Nantes, France5

Received 27 January 2011/Returned for modification 30 July 2011/Accepted 8 October 2011

Pertussis is routinely diagnosed with real-time PCR based on insertion sequence IS481, which is not specific for *Bordetella pertussis*. We conducted a retrospective study using real-time PCRs specific for *Bordetella pertussis* and for *Bordetella holmesii* on 177 samples positive for IS481 PCR. *Bordetella holmesii* DNA was detected in 20.3% samples collected from adolescents and adults.

*Bordetella holmesii* is known to be responsible for bacteremia in hyposplenic patients, including those affected by sickle cell anemia, and has also been isolated from the sputum of patients with pertussis symptoms (4, 6, 10). The diagnosis of *Bordetella* infections routinely involves real-time PCR (9). Two insertion sequences, IS481 and IS1001, are commonly used as PCR targets because numerous copies are present in the bacterial genomes and this contributes to the sensitivity of these tests. However, (i) IS481 is present in the genome of *B. holmesii* isolates and some *B. bronchiseptica* isolates and is therefore not specific for *B. pertussis* and (ii) IS1001 is present in the genome of some *B. bronchiseptica* and is therefore not specific for *B. parapertussis* (9).

The aim of this retrospective study was to identify the *Bordetella* species in biological samples positive for IS481 collected between 2009 and 2010 in four French laboratories. To identify the species, we used previously developed specific “in-house” real-time PCR. These real-time PCRs are based on the amplification of the promoter of the pertussis toxin operon (ptxA-Pr-based PCR) specific for *B. pertussis* (1) and of the recA gene (RecA-based PCR) specific for *B. holmesii* (2). We also used a real-time PCR based on the BP3385 gene (BP3385-based PCR), which was initially described as specific for *B. pertussis* (5) but was subsequently shown to score positive for some *B. bronchiseptica* isolates (8). This PCR can replace the ptxA-Pr-based PCR to detect *B. pertussis* carrying a deletion of the whole ptx operon, which is pertinent because such *B. pertussis* isolates can circulate (3). We also used the IS1001 PCR to analyze coinfections (9). The primers used to perform the “in-house” PCRs are listed in Table 1.

The analytical sensitivity of the different assays was determined by using a series of 10-fold dilutions of *B. pertussis* Tohama, *B. parapertussis* 12822, and *B. holmesii* BHO1 DNAs (7, 9). Each dilution was tested three times independently. The limits of detection per PCR in our conditions were 0.5 CFU and 1 CFU for IS481- and IS1001-based PCRs, respectively, using the Argene kits (catalog no. 69-0011B for IS481-based PCR and 71-012 for IS1001-based PCR; Argene, Verniolle, France), 30 CFU for the in-house ptxA-Pr-based PCR, 30 CFU for the in-house BP3385-based PCR, and 50 CFU for the in-house RecA-based PCR. Because of the difference of sensitivity between the routine IS481- and IS1001-based PCRs and the specific in-house PCRs we decided to analyze only biological samples with a threshold cycle (Ct) of <30 as assessed with IS481 PCR. We selected 177 biological samples from nasopharyngeal aspirates or swabs from suspected cases of pertussis. DNA was extracted using the High Pure PCR template preparation kit (Roche), the EZ1 DNA tissue kit (Qiagen), or easyMAG (bioMérieux). The Ct values obtained with the IS481 PCR for these 177 samples were all between 8 and 30. We performed the four PCRs on all samples.

A total of 163 (92%) samples scored positive by ptxA-Pr-based PCR and BP3385-based PCR and negative by RecA-based PCR and IS1001-based PCR and were therefore determined to contain *B. pertussis* DNA.

One (0.6%) sample was positive by ptxA-Pr-based PCR, BP3385-based PCR, and IS1001-based PCR and negative by RecA-based PCR and was therefore determined to contain *B. pertussis* and *B. parapertussis* or *B. bronchiseptica* DNA.

A total of 12 (6.8%) samples scored positive by RecA-based PCR and negative by ptxA-Pr-based PCR, BP3385-based PCR, and IS1001-based PCR and were therefore determined to contain *B. holmesii* DNA.

Two (1.1%) samples were ptxA-Pr-based PCR negative but positive by BP3385-based PCR. These samples may contain DNA not carrying the ptx operon as previously described (3) or a quantity of DNA below the limit of detection of the ptxA-Pr target or *B. bronchiseptica* DNA.

No sample was both positive for the ptxA-Pr-based PCR and negative for the BP3385-based PCR.

Information concerning birth date was available for the 177
patients. We defined three groups: 0 to 9 (n = 119), 10 to 17 (n = 20), and 18 or more years old (n = 39). Among the 59 patients older than 9 years, 20.3% were identified as being infected with \textit{B. holmesii} and not \textit{B. pertussis}; 6 of these cases were between 10 and 17 years of age (30% of the cases analyzed in the age group) and 6 were between 18 and 48 years of age (15.3% of the analyzed cases in this age group). Although the number of samples analyzed is small, these findings demonstrate that \textit{B. holmesii} is frequently present in biological samples from adolescents and adults who have respiratory symptoms clinically diagnosed as pertussis. Generally, this species is isolated in blood cultures of immunocompromised or asplenic patients but also, although less frequently, in patients with pertussis syndrome (9). This is consistent with previous reports describing \textit{B. holmesii} infection in patients with pertussis-like symptoms (4, 6–10). Surprisingly, no patient under 9 years of age was carrying \textit{B. holmesii} DNA, suggesting a lower rate of transmission of \textit{B. holmesii} than of \textit{B. pertussis} from adults to infants. All samples collected from infants or young children were confirmed as containing \textit{B. pertussis}, and only one case of co-infection (\textit{B. pertussis}-\textit{B. parapertussis} or \textit{B. bronchiseptica}) was observed (a 5-year-old patient). The two patients confirmed as carrying either \textit{B. pertussis} or \textit{B. bronchiseptica} were 1 and 60 years of age. Because no information is available concerning the vaccine status or animal contacts of these two patients, conclusions are difficult to formulate.

In conclusion, we confirm that \textit{B. holmesii} is indeed a bacterial species associated with pertussis-like symptoms: 20% of patients (12 patients over 9 years of age) previously identified as having pertussis were infected with \textit{B. holmesii}. No coinfection with \textit{B. pertussis} and \textit{B. holmesii} was found. The number of samples we tested is small, but the proportion of \textit{B. holmesii}-positive samples was higher than previously described (2). In our study, all cases of \textit{B. holmesii} detection were in adolescents and adults and not in infants. At present, we do not know whether \textit{B. holmesii} is either a human respiratory pathogen or an opportunistic bacterium carried mostly by adolescents and adults and detected only during an episode of cough due to another \textit{Bordetella} infection or another microorganism. We do not know whether this phenomenon is new and due to the decrease in the incidence of pertussis, following the introduction of pertussis boosters, or one that has been simply overlooked in the past. Before 2005, only the \textit{B. pertussis}-specific PCR was routinely used in France. Since then, most laboratories routinely use the IS481-based PCR because of its high sensitivity, and it is therefore difficult to choose between the two hypotheses. Due to the limitations of retrospective investigation, this study is unable to confirm the presence of \textit{B. holmesii} by culture. It is, however, very important to emphasize that isolation of this organism would strengthen the recognition of \textit{B. holmesii} and its potential clinical and epidemiological implications in whooping cough-like illness.

Surveillance of \textit{B. holmesii} should be pursued, and all clinicians and epidemiologists need to be aware of this observation. Indeed, correct identification using specific real-time PCR for each \textit{Bordetella} species and culture of this pathogen is important for the active surveillance of \textit{Bordetella} infections in the whole population, particularly in adolescents and adults.

This work was performed with the financial help of the Institut Pasteur Foundation, CNRS-URA3012, and of the Institut de Veille Sanitaire.

### REFERENCES


---

**TABLE 1. Primers and probes used for the real-time PCR**

<table>
<thead>
<tr>
<th>PCR</th>
<th>Primer or probe</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ptxA-Pr-based PCR</td>
<td>PT1</td>
<td>5′-CCA ACG CGC ATG CGT GCA CAT TCG TC-3′</td>
</tr>
<tr>
<td></td>
<td>PT2</td>
<td>5′-CCC TCT CGG TTT TGA TGG TGC CTA TTT TA-3′</td>
</tr>
<tr>
<td></td>
<td>PT-FAM</td>
<td>Fam-AAT CCA ACA CGG CAT GAA CGC TCC TT-BHQ</td>
</tr>
<tr>
<td>RecA-based PCR</td>
<td>HrecAF</td>
<td>5′-CCG AAT CCT CGG AGA C-3′</td>
</tr>
<tr>
<td></td>
<td>HrecAR</td>
<td>5′-GCT GTG GCG TTT TGA GAT G-3′</td>
</tr>
<tr>
<td></td>
<td>HrecAF-FAM</td>
<td>eFAM-CAC TTT GTC CTT GTC CGT CAG TGC TGA G-BHQ</td>
</tr>
<tr>
<td>BP385-based PCR</td>
<td>BP385-fwd</td>
<td>5′-gTTTTTCTAAGCGCTTAATG-3′</td>
</tr>
<tr>
<td></td>
<td>BP385-rev</td>
<td>5′-TGTCTCCGACATTTGTTTATG-3′</td>
</tr>
<tr>
<td></td>
<td>BP385-FAM</td>
<td>eFAM-CgACAggCTCTACCAACgCgCT CT-BBQ</td>
</tr>
</tbody>
</table>

**PCR Primer or probe**

**Sequence**

**TABLE 1. Primers and probes used for the real-time PCR**