Significant finding of *Bordetella holmesii* DNA in nasopharyngeal samples from French patients with suspected pertussis

Elisabeth Njamkepo¹,², Stéphane Bonacorsi³, Monique Debruyne⁴, Sophie Anne Gibaud⁵, Sophie Guillot¹,² and Nicole Guiso¹,²*

Running title: *Bordetella holmesii* DNA in nasopharyngeal samples

¹ Institut Pasteur, Molecular Prevention and Therapy of Human Diseases, National Centre of Reference of Whooping cough and other bordetelloses
² CNRS-URA 3012, Paris France
³ Hôpital Robert Debré, Service de Microbiologie, Paris France
⁴ Laboratoire Cerba, Saint Ouen L’Aumône, France
⁵ Laboratoire de Bactériologie, Centre Hospitalier Universitaire de Nantes, Nantes, France

*Corresponding author*: Institut Pasteur, Unité de Prévention et Thérapies Moléculaires des Maladies Humaines, CNRS-URA 3012, 25 rue du Dr Roux 75724 Paris, cedex 15, France; Tel: 00 +33 01 45 68 83 34; e-mail: nicole.guiso@pasteur.fr

Mot clés : *Bordetella holmesii; Bordetella pertussis*; nasopharyngeal samples; DNA; PCR.
Abstract

Pertussis is routinely diagnosed with real-time PCR based on Insertion Sequences 481 which is not specific for *Bordetella pertussis*. We conducted a retrospective study using real-time PCR specific for *Bordetella pertussis* and for *Bordetella holmesii*, on 177 samples positive with IS481 PCR. *Bordetella holmesii* DNA was detected in 20.3% samples collected on adolescents and adults.

Words 55
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 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 and is therefore not specific for B. pertussis (ii) IS1001 is present in the genome of some B. bronchiseptica isolates 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 PCR 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 co-infections (9). The primers used to perform the “in-house” PCRs are listed Table 1.

The analytical sensitivity of the different assays was determined by using 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 (Ref. 69-0011B for IS481-based PCR and ref. 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 Ct <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™ (Biomerieux). The Ct obtained with the IS481 PCR for these 177 samples were all between 8 and 30. We performed the four PCRs on all samples:

163 (92%) samples scored positive by ptxA-Pr-based PCR and BP3385-based PCR and negative by RecA-based PCR and IS1001-based PCR, contained therefore B. pertussis DNA

1 (0.6%) sample was positive by ptxA-Pr-based PCR, BP3385-based PCR and IS1001-based PCR and negative by RecA-based PCR, contained therefore B. pertussis and B. parapertussis DNA

12 (6.8%) samples scored positive by RecA-based PCR and negative by ptxA-Pr-based PCR, BP3385-based PCR and IS1001-based PCR, contained therefore B. holmesii DNA

2 (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 positive for the ptxA-Pr-based PCR and negative for the BP3385-based PCR.
Information concerning the birth date was available for the 177 patients. We defined three
groups: 0-9 (119), 10-17 (20) and 18 or more years old (39). Among the 59 patients older than
9 years, 20.3% were identified as being infected with *B. holmesii* and not *B. pertussis*; six of
these cases were between 10 to 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 *B.
holmesii* is frequent in biological samples from adolescents and adults who have respiratory
symptoms clinically diagnosed as pertussis. Generally, this species is isolated in blood culture
of immuno-compromised or asplenic patients but also, although less frequently, in patients
with pertussis syndromes (9). This is consistent with previous reports describing *B. holmesii*
infection in patients with pertussis-like symptoms (4, 6, 10). Surprisingly, no patient under 9
years of age was carrying *B. holmesii* DNA, suggesting lower transmission of *B. holmesii* than
of *B. pertussis* from adults to infants. All samples collected from infants or young children
were confirmed as containing *B. pertussis* and only one case of co-infection (*B. pertussis/B.parapertussis*)
was observed (a 5 year-old patient). The two patients confirmed as
carrying either *B. pertussis* or *B. bronchiseptica* were 1 and 60 years of age. No information is
available concerning the vaccine status or animal contacts of these two patients, conclusions
are difficult.

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

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

**Acknowledgments**

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


Table 1: Primers and probes used for the RT-PCR

<table>
<thead>
<tr>
<th>PCR</th>
<th>Primers and probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ptxA-Pr based</td>
<td>PT1 : 5’-CCA ACG CGC ATG CGT GCA GAT TCG TC-3’</td>
</tr>
<tr>
<td></td>
<td>PT2 : 5’-CCC TCT GCG TTT TGA TGG TGC CTA TTT TA-3’</td>
</tr>
<tr>
<td></td>
<td>PT-FAM : Fam-AAT CCA ACA CGG CAT GAA CGC TCC TT-BHQ</td>
</tr>
<tr>
<td>RecA-based PCR</td>
<td>HrecAF : 5’-CCG AAT CCT CGG GCA AGA C-3’</td>
</tr>
<tr>
<td></td>
<td>HrecAR : 5’-GGT GTC CGG TTG GGA GAT -3’</td>
</tr>
<tr>
<td></td>
<td>RecA-FAM : 6FAM-CAC TTG TGC CTT CGT CGA TGC TGA G-BBQ</td>
</tr>
<tr>
<td>BP3385-based</td>
<td>BP3385-fwd : 5’ ggTTTCTTTCAggCCCTAAATgg-3’</td>
</tr>
<tr>
<td>PCR</td>
<td>BP3385-rev : 5’ TCgTCCTCgACgTgTgTgTAg-3’</td>
</tr>
<tr>
<td></td>
<td>BP3385-Fam : 6FAM-CgACAggCTCTACCAAACgCgCTCT-BBQ</td>
</tr>
</tbody>
</table>