

# Emergence of *Bordetella holmesii* as a Causative Agent of Whooping Cough, Barcelona, Spain

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We describe the detection of *Bordetella holmesii* as a cause of whooping cough in Spain. Prevalence was 3.9% in 2015, doubling to 8.8% in 2016. This emergence raises concern regarding the contribution of *B. holmesii* to the reemergence of whooping cough and the effectiveness of the pertussis vaccine.

Whooping cough is a highly contagious respiratory disease, primarily caused by *Bordetella pertussis* (1). Other species, such as *B. parapertussis* and *B. holmesii*, have been recognized as causes of a syndrome that clinically resembles that of whooping cough (1,2). Pertussis is the term used for the disease specifically caused by *B. pertussis*, whereas pertussis-like illness or syndrome is more appropriately used when referring to the other etiologic agents. *B. holmesii*, a poorly studied pathogen, was originally identified in 1995 as a rare cause of bacteremia (3). Since then, it has been related to other invasive diseases, especially in asplenic and immunosuppressed patients and in healthy people with pertussis-like symptoms (4).

Microbiologic diagnosis of whooping cough by molecular tests provides a higher sensitivity and promptness than culture techniques, with PCR being the method most commonly used in clinical laboratories (5). Most molecular diagnostic kits used to detect *B. pertussis* target insertion sequence IS481, which is present in high copy numbers in the *B. pertussis* genome (6). However, IS481 is not a

specific target of *B. pertussis* because it is also found in other *Bordetella* species, including *B. holmesii*, leading to underestimation of this pathogen in this clinical scenario (6).

To date, several cases of *B. holmesii* associated with pertussis-like illness have been reported in North and South America, Asia, Africa, and Europe (4). Additionally, 2 important outbreaks of *B. holmesii* infection associated with pertussis-like illness were detected in France and Ohio (7,8). Recent reports of the detection of positive cases of *B. holmesii* infection in the Netherlands (9), which previous analysis had failed to identify (10), reinforce the emergence of this pathogen. To our knowledge, the presence of this microorganism in Spain has not been documented. We report the emergence of *B. holmesii* as a causative agent of whooping cough in the metropolitan area of Barcelona, Spain.

## The Study

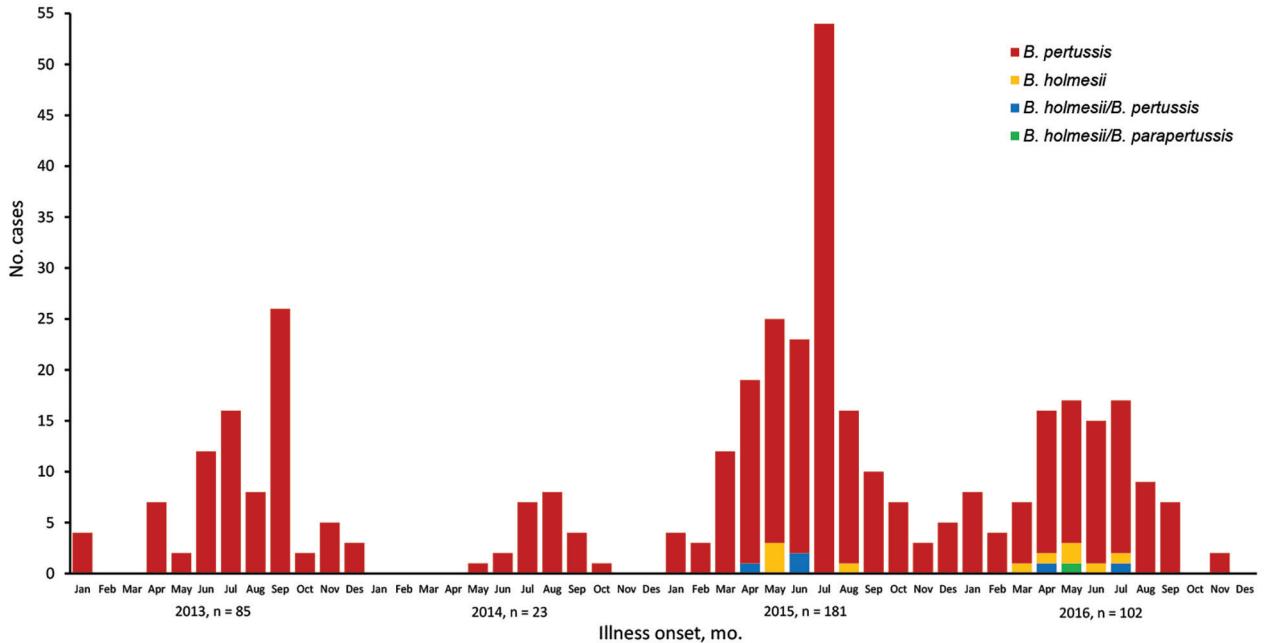
We evaluated 391 nasopharyngeal samples from patients from the metropolitan area of Barcelona who had a clinical and laboratory-confirmed diagnosis of whooping cough during January 2013–December 2016 at the Hospital Vall d'Hebron. All the samples were positive by the IS481-based SmartBp/Bpp (Cepheid, Sunnyvale, CA, USA) real-time PCR and thus were considered positive for *B. pertussis*.

We reevaluated all the samples by using species-specific multiplex real-time PCR (10). This method detects the promoter of the pertussis toxin operon (*ptxAPr*), which is specific for *B. pertussis*, and the *recA* gene (*Bh-RecA*), specific for *B. holmesii*. To corroborate the identification of *B. holmesii*, we further analyzed all the *Bh-RecA* RT-PCR-positive samples by sequencing an internal fragment of the housekeeping gene encoding the ribonucleoside-diphosphate reductase  $\alpha$  chain (*nrdA*), which is useful for discriminating among the different species of *Bordetella* (11), and the *Bh-RecA* gene. The study was approved by the Clinical Research Ethics Committee of the hospital.

Among the 391 nasopharyngeal samples analyzed, 380 (97.2%) were confirmed positive for *B. pertussis* and 16 (4.1%) for *B. holmesii*. Among the *B. holmesii*-positive samples, 5 were positive for *B. pertussis* and *B. holmesii* and 1 for *B. parapertussis*, *B. holmesii*, and *Streptococcus pyogenes* (Figure).

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**Figure.** Timeline distribution of the 391 whooping cough cases diagnosed at the Hospital Vall d'Hebron, Barcelona, Spain, 2013–2016, showing *Bordetella* species detected.

None of the *B. holmesii*-positive cases was detected during 2013–2014. In total, 7 cases were reported in 2015, corresponding to 3.9% of whooping cough cases diagnosed in 2015, and the remaining 9 cases were reported in 2016, accounting for 8.8% of the cases diagnosed during that year (Figure).

Ten (62.5%) of the 16 *B. holmesii*-positive patients were female; the median age was 9 years (range 1–40 years), and 87.5% were pediatric patients (<14 years). Fourteen cases were detected in the context of a school-related (85.7%) or family (35.7%) outbreak; 3 of these cases were detected in both kinds of outbreaks.

Vaccination status was available for 14 of the 16 patients. Of these, all cases occurred in children 14 months to 14 years of age who had received a median of 5 doses of pertussis vaccine (range 2–5 doses) according to the current vaccination program (5 doses, administered at 2, 4, and 6 months and at 1.5 and 6 years of age). The median time since the last vaccination was 4.5 years (range 0.7–14.1 years) (Table 1). No cases of complications or malignant pertussis-like disease occurred. Information about antimicrobial therapy received was available for 15 patients, all of whom had been treated with

**Table 1.** Demographic, clinical, and epidemiologic characteristics of 16 patients with diagnosed whooping cough associated with *Bordetella holmesii* infection, Hospital Vall d'Hebron, Barcelona, Spain, 2015–2016\*

| Patient no. | Age, y/sex | No. vaccine doses received | Date last vaccine dose received | Diagnosis date | Treatment | Co-infections                                   | Outbreak relatedness | Site of exposure |
|-------------|------------|----------------------------|---------------------------------|----------------|-----------|---|----------------------|------------------|
| 1           | 10/F       | 5                          | 2010 Mar 16                     | 2015 Apr 17    | AZM       | <i>B. pertussis</i>                             | Yes                  | School           |
| 2           | 12/F       | 5                          | 2008 Jul 18                     | 2015 May 5     | AZM       | ND  | Yes                  | School†          |
| 3           | 9/F        | 5                          | 2011 Dec 12                     | 2015 May 13    | AZM       | ND  | Yes                  | School†          |
| 4           | 13/F       | 5                          | 2007 Oct 23                     | 2015 May 25    | AZM       | ND  | Yes                  | School†          |
| 5           | 12/F       | 5                          | 2008 Sep 6                      | 2015 Apr 6     | AZM       | <i>B. pertussis</i>                             | Yes                  | School           |
| 6           | 28/F       | UNK                        | UNK                             | 2015 Apr 6     | AZM       | <i>B. pertussis</i>                             | Yes                  | Home             |
| 7           | 4/F        | 4                          | 2012 Mar 8                      | 2015 Aug 14    | AZM       | ND  | Yes                  | School and home  |
| 8           | 9/M        | 5                          | 2012 Oct 18                     | 2016 Sep 3     | UNK       | ND  | Yes                  | School           |
| 9           | 1/M        | 2                          | 2015 Jun 8                      | 2016 April 13  | AZM       | <i>B. pertussis</i>                             | Yes                  | Home             |
| 10          | 8/M        | 5                          | 2012 Jun 26                     | 2016 Apr 21    | AZM       | ND  | Yes                  | School and home  |
| 11          | 6/M        | 4                          | 2011 Aug 8                      | 2016 Mar 5     | AZM       | <i>B. parapertussis</i> /<br><i>S. pyogenes</i> | Yes                  | School           |
| 12          | 40/F       | UNK                        | UNK                             | 2016 Sep 5     | AZM       | ND  | UNK                  | UNK              |
| 13          | 14/F       | 3                          | 2002 Aug 5                      | 2016 May 24    | AZM       | ND  | No                   | –                |
| 14          | 5/F        | 4                          | 2012 Mar 2                      | 2016 Sep 6     | AZM       | ND  | Yes                  | School and home  |
| 15          | 9/M        | 5                          | 2013 Sep 10                     | 2016 Nov 7     | AZM       | ND  | Yes                  | School           |
| 16          | 6/M        | 4                          | 2011 Feb 16                     | 2016 Jul 28    | AZM       | <i>B. pertussis</i>                             | Yes                  | School           |

\*AZM, azithromycin; ND, not detected; UNK, unknown.

†These 3 patients' illnesses were related to the same school outbreak.

**Table 2.** Comparison of demographic, vaccination-related, and clinical characteristics between patients with *Bordetella pertussis* and *B. holmesii* infection, Hospital Vall d'Hebron, Barcelona, Spain, 2015–2016\*

| Characteristic  | <i>B. pertussis</i> , n = 40 | <i>B. holmesii</i> , n = 10 | p value |
|---|------------------------------|-----------------------------|---------|
| Median age (range), y   | 5.5 (0.08–74)                | 9 (4–40)                    | 0.07    |
| Median pertussis vaccine doses received (range)                                       | 4 (0–5)                      | 5 (3–5)                     | 0.21    |
| Median time from last pertussis vaccine dose received to date of diagnosis (range), y | 1.92 (0.08–11.70)            | 3.82 (1.03–14.05)           | 0.1     |
| Fever, no. (%)  | 5 (12.5)                     | 1 (10)                      | 1       |
| Whoop, no. (%)  | 9 (22.5)                     | 1 (10)                      | 0.66    |
| Paroxysms, no. (%)  | 4 (10)                       | 1 (10)                      | 1       |
| Cough ≥14 d, no. (%)  | 12 (30)                      | 4 (40)                      | 0.7     |
| Hospitalized, no. (%)   | 4 (10)                       | 0                           | 0.57    |

\*Differences were assessed for significance using the chi-squared exact test (in comparison with independent qualitative variables) and the Mann-Whitney *U*-test (for quantitative variables; no normality was observed in data distribution). We selected a randomized sample of confirmed *B. pertussis* cases with a 4:1 relation with *B. holmesii*-infected patients as a comparison group. p values <0.05 were considered statistically significant at the 95% CI level.

azithromycin, and no patient experienced therapeutic failure or relapse.

No statistical differences were observed between age, clinical features, and vaccination status among the case-patients with *B. holmesii* and *B. pertussis* infections (Table 2). However, *B. holmesii* infections tended to be more prevalent in older children (median age 9 vs. 5.5 years;  $p = 0.07$ ) compared with *B. pertussis* infections.

## Conclusions

*B. holmesii* is an underdiagnosed emerging respiratory pathogen that triggers clinical manifestations similar to those caused by *B. pertussis* (1). In this retrospective study, we detected 10 cases in which *B. holmesii* was found to be the only putative agent of a pertussis-like infection and 6 cases in which *B. holmesii* was co-detected with another causative agent of whooping cough. We observed no differences in the demographics, clinical features, and vaccination status among patients infected by *B. holmesii* and *B. pertussis*, but a trend toward higher involvement of *B. holmesii* infections was observed in older children, as reported previously (7,8).

We found that 4.1% of the respiratory samples from patients with laboratory-confirmed whooping cough during 2013–2016 were positive for *B. holmesii*, for which detection was reported from April 2015 onward. The number of positive cases of *B. holmesii* infection doubled from 3.9% in 2015 to 8.8% in 2016. Of note, 2015 was considered the year with the highest incidence of whooping cough since the introduction of the acellular vaccine in Spain. In the autonomous community of Catalonia, incidence (cases/100,000 inhabitants) was 13.3 for 2013, 14.8 for 2014, 48.9 for 2015, and 24.6 for 2016 ([http://canalsalut.gencat.cat/ca/actualitat/llista\\_butlletins/salut\\_publica/butlleti\\_epidemiologic\\_de\\_catalunya](http://canalsalut.gencat.cat/ca/actualitat/llista_butlletins/salut_publica/butlleti_epidemiologic_de_catalunya)).

Even in the absence of clear recommendations to treat pertussis-like respiratory infections caused by *B. holmesii*, several studies have reported controversial results about a possible lower activity of macrolides

compared with other antimicrobial agents (4,12). Unfortunately, because we could not recover the bacterial isolates, we were unable to perform antimicrobial drug susceptibility testing. However, no evidence of complications or relapses was observed in any patient after treatment with azithromycin.

*B. holmesii* lacks most of the antigens present in the pertussis acellular vaccine or the proteins produced differ phenotypically (4). This situation, together with the lack of protection against replication observed in immunized mice (13), suggests the absence of cross-protection against *B. holmesii* infections. In our study, most of the patients had received the complete immunization schedule of 5 doses (Table 1). Thus, the increasing trend of whooping cough might be attributed not only to *B. pertussis* adaptation to the introduction of the acellular pertussis vaccine, decreased vaccine efficacy, or waning immunity, as previously reported (14,15), but also to the emergence of secondary pathogens, such as *B. holmesii*, which the pertussis vaccine might not prevent.

Our study describes the emergence of *B. holmesii* as a causative agent of whooping cough in Spain. Accurate diagnosis of the causative agent of this disease is crucial to determine the real incidence and prevalence of the microbial species involved, to assess its contribution to the epidemiology of whooping cough, to evaluate whether specific antimicrobial drug treatments should be implemented and, in terms of public health, to assess the efficacy of the pertussis vaccine.

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Ms. Cros-Mir is a PhD student working at the Microbiology Group of the Hospital Vall d'Hebron Research Institute, Barcelona, Spain. Her research interests are the epidemiology and molecular characterization of *Bordetella pertussis* and other related species.

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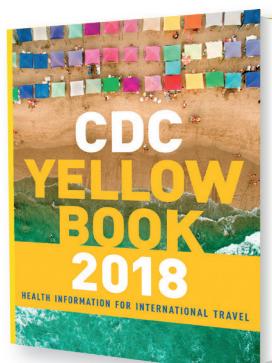
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