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## **Real-life requests for *Bordetella* polymerase chain reaction testing in children presenting to hospital**

Short title: *Bordetella* PCR requests in real-life hospital setting

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**Abstract**

From 2015 to 2017, 3197 interpretable *Bordetella* polymerase chain reaction (PCR) tests were performed for 2760 children presenting to our tertiary university hospital. Requests mainly came from the emergency department (62%) and for children older than 1 year (68%). Only 32 PCR (1%) results were positive, mainly in children younger than 1 year ( $n=29/32$ , 90.6%;  $p<0.001$ ). When focusing on the PCR indications in 2017, we found the requests were mainly based on nonspecific respiratory symptoms and were clinically unjustified in 383 cases (39%). Pediatricians overused *Bordetella* PCR in clinical practice. They should reserve their requests for cases of young children with symptoms suggestive of respiratory illness and/or incomplete pertussis immunization.

**Keywords:** pertussis; children; polymerase chain reaction.

## 1 Introduction

Pertussis, mainly due to *Bordetella pertussis*, is a respiratory disease responsible for more than 200,000 deaths per year worldwide, with the high-risk population being neonates and young infants [1–3]. The *Bordetella* real-time polymerase chain reaction (PCR), performed on nasopharyngeal samples within the 3 first weeks of the disease, is the current validated diagnostic method of choice, with a better sensitivity and specificity compared to culture and serology [4]. To our knowledge, there is no study on the real-life pertinence of *Bordetella* PCR in children presenting to a tertiary university hospital and the clinical reasons for the PCR. Our aim was to characterize the positivity rate of PCR in this setting in real life and identify the clinical signs for its request.

## 2. Material and methods

### 2.1. Study design

This retrospective study was conducted with patients younger than 15 years presenting to the hospitals of the *Assistance Publique – Hôpitaux de Marseille* (AP-HM) in France between January 1, 2015 and December 31, 2017. To note, the AP-HM is composed of three tertiary university hospitals including pediatric emergency departments, neonatology units, general and specialized pediatric units, and pediatric intensive care units. Patients, who were treated in one of the aforementioned wards, were identified from the microbiological laboratory records after nasopharyngeal samples were taken for *Bordetella* PCR. For each *Bordetella* PCR request, we collected data such as the age and gender of the patient, the date of sampling, the requesting pediatric unit, and the PCR result. A focused analysis was conducted in 2017 to collect additional data: vaccine status against pertussis, clinical symptoms resulting in the PCR request, including typical signs of pertussis (cough with paroxysmal episodes, or with emesis, or with apnea), and final diagnosis at discharge.

## 2.2. Microbiological data

The PCR was carried out in the same microbiological laboratory [5]. DNA was automatically extracted from respiratory samples using the EZ1 advanced XL Bacteria Card (Qiagen, Courtaboeuf, France) through a 200- $\mu$ l test portion. Following this, 50  $\mu$ l of purified DNA was used to amplify the *Bordetella pertussis* genome with primers targeting the *IS481* gene (also detecting *Bordetella holmesii*), with a detection threshold of 145 CFU/mL and 152 CFU/mL for *Bordetella pertussis* and *holmesii*, respectively [6]. PCR was conducted using a CFX-96 system (Bio-Rad, Marnes-la-Coquette, France). The results were obtained after a 1.5-h run. To make sure the sample was extracted correctly we performed PCR analysis of the beta-globin gene. If this result was negative, *Bordetella* PCR was concluded to be uninterpretable. Each PCR test was analyzed as a single sample, even if the PCR tests were repeated for the same patient on different dates. The cost of the analysis was 39 euros per sample.

## 2.3. Statistical analysis

Variables are expressed in absolute values for quantitative data and in percentage for qualitative data. The chi-square test was used to find statistically significant links between sex, age range, and positivity. Values of  $p < 0.05$  were considered statistically significant.

## 2.4. Ethical considerations

Authorization was obtained from the informatics and liberty committee (CNIL 2018-38) and from the ethics committee of the Pediatric French Society (CER\_SFP\_2018\_083-2). Each family was informed about the study.

# 3. Results

## 3.1. Study period 2015–2017

Within the 3-year study period, 3258 *Bordetella* PCR tests were performed on nasopharyngeal samples, with 2022 requests (62%) coming from the emergency departments. Among them, 61 cases were excluded from our study (42 not analyzed, 18 not interpretable, 1 doubtful). Therefore, 3197 analyses for 2760 patients (13.6% multiple testing) were included. The mean age of the patients was 21 months, with a sex ratio of 1.15 (1477 males). Approximately one third of the samples (1152/3197, 32%) were from infants aged less than 1 year. Only 32 positive PCR results (1%) were noted during the 3-year period (**Figure 1**), mainly in children younger than 1 year (90.6%,  $p < 0.001$ ).

### 3.2. Focus on 2017

In 2017, there were 1073 *Bordetella* PCR requests for 905 patients, 92 being excluded (24 not analyzed by the laboratory, 4 not interpretable after analysis, 64 with missing medical information). A total of 19 PCR test results were positive among the 981 requests (1.9%) accounting for 828 patients. The same particularities were noted as with the 3-year period, with a positive PCR result in only 19 children, including 18 of whom were younger than 1 year ( $p = 0.01$ ). The main symptoms justifying the PCR requests in 2017, including those with a positive result, are listed in **Table 1**. Approximately 80% of the PCR tests with positive results were performed because of a cough ( $n = 15$ ), with a clinically typical form of whooping cough in 11 cases (57.9%). In brief resolved unexplained events with cyanosis (apnea), PCR results were positive in four cases, all of them being associated with a cough. *Bordetella* PCR tests requested because of a brief resolved unexplained event were all negative when respiratory symptoms were absent. No PCR result was positive when this event was accompanied by pallor. The vaccine status was recorded for 739 cases (75%), with an up-to-date status in 638 (86.8%). Most cases of pertussis occurred in children who did not receive their three vaccine injections (16/18, missing data in two cases). Only one 15-month-old patient developed pertussis despite three delayed injections. Diagnosis at discharge was

missing in the files of 209 PCR requests (21.3%). When present, the final diagnosis included upper respiratory tract infections (211 cases; 21.5%), lower respiratory tract infections (191 cases; 19.5%), asthma (78 cases; 7.9%), severe manifestations such as acute respiratory distress syndrome, septic shock, cardiac arrest, or *Pneumocystis* infection (12 cases; 1.2%). Ten patients (accounting for 11 PCR requests) were considered as probably having pertussis, even though the PCR results were negative. All 828 patients received antibiotic treatment, regardless of the PCR results. When considering a *Bordetella* PCR request to be inappropriate in clinical situations such as febrile neutropenia, asthma exacerbation, pre-graft and pre-surgery check-up, bronchiolitis obliterans, PCR follow-up after antibiotic treatment, flu, gastroesophageal reflux, and physiological manifestations, a minimum of 383 prescriptions (39%) could have been avoided.

#### **4. Discussion**

We found that clinicians commonly requested *Bordetella* PCR, and often for irrelevant indications. A PCR positivity rate of only 1% was found during the 3-year study period. Of course, our study has some limitations. Several patients were subjects to multiple testing, responsible for analytic inaccuracy. As data were collected retrospectively, some PCR requests were excluded because of missing information, and the pertussis vaccine status of all children who were tested was missing. The low number of positive PCR results was also a limitation, responsible for a low statistical power. Infections due to *Bordetella parapertussis* were not sought for, and those due to *Bordetella holmesii*, which have been described with a very high prevalence in France in patients with pertussis-like symptoms [7], could not be differentiated from infections due to *Bordetella pertussis* with our PCR tests. Therefore, we show an excessively high request rate for PCR in pediatric cases presenting to a tertiary university hospital in real life, perhaps due to the easy access at our point of care. To

compare, a previous work conducted in Bordeaux found a PCR positivity rate of 14.3% in infants [8]. Considering the cost of the PCR technique, it would be economically beneficial to reduce the number of samples to more relevant indications. Cough is a shared symptom of all upper and lower respiratory tracts, whatever the cause, but the absence of fever and a cough that lasts more than 1 week with paroxysmal episodes and/or cough-induced emesis are more suggestive of pertussis [9]. However, all cases of cough in unvaccinated or incompletely vaccinated infants should be considered as pertussis, even in the case of positive viral identification. In the absence of respiratory symptoms, including apnea, which could be the sole sign of pertussis, PCR requests appear to be irrelevant, especially in the case of brief resolved unexplained events. New recommendations regarding brief resolved unexplained events, aiming to avoid useless complementary examinations, consider that *Bordetella* PCR is justified in this indication only for children at risk, with incomplete immunization, or presenting with suggestive symptoms [10]. Our results also question the relevance of *Bordetella* PCR requests in children older than 1 year, where complex or severe clinical situations in which the diagnosis remains challenging could be the best indications. Interestingly, no PCR request for screening of close contacts of an infant suspected of having pertussis, as is usually recommended, was noted in our study.

## **5. Conclusion**

The global positivity rate of *Bordetella* PCR appears to be dramatically low in real-life pediatric hospital practice, partly due to unjustified requests for PCR, in particular among children older than 1 year. Clinicians should reserve their requests to cases of infants under 1 year of age with respiratory symptoms such as cough, apnea, or a clinically typical form of whooping cough, especially when there is incomplete immunization.

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**Table 1:** Rate of *Bordetella* PCR positivity depending on clinical symptoms of 828 children aged younger than 15 years presenting in 2017 to the three tertiary university hospitals of the *Assistance Publique – Hôpitaux de Marseille*, France (19 positive PCRs)

| Symptoms  | Positive<br><i>Bordetella</i> PCRs | Negative<br><i>Bordetella</i> PCRs | Positivity rate<br>(%) |
|---|------------------------------------|------------------------------------|------------------------|
| Typical signs of pertussis +/- apnea                    | <i>n</i> =11                       | <i>n</i> =14                       | 44                     |
| Unspecified cough +/- respiratory distress              | <i>n</i> =5                        | <i>n</i> =535                      | 0.9                    |
| Unspecified cough and wheezing +/- respiratory distress | <i>n</i> =2                        | <i>n</i> =153                      | 1.3                    |
| Unspecified cough > 3 weeks                             | <i>n</i> =0                        | <i>n</i> =31                       | 0                      |
| Isolated respiratory distress                           | <i>n</i> =1                        | <i>n</i> =30                       | 3.2                    |
| Brief resolved unexplained event without cyanosis       | <i>n</i> =0                        | <i>n</i> =77                       | 0                      |
| Brief resolved unexplained event with pallor            | <i>n</i> =0                        | <i>n</i> =28                       | 0                      |
| Unexplained crying                                      | <i>n</i> =0                        | <i>n</i> =14                       | 0                      |

PCR: polymerase chain reaction

**Figure 1:** Distribution of *Bordetella* PCR test results by age of 2760 children tested between 2015 and 2017 at the *Assistance Publique–Hôpitaux de Marseille*, France, with only 32 positive cases.

PCR: polymerase chain reaction

