Clinical characteristics and molecular detection of *Bordetella pertussis* in hospitalized children with a clinical diagnosis of whooping cough in Peru

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**ABSTRACT**

**Background and Objectives:** Pertussis is an infectious disease caused by the Gram-negative bacterium *Bordetella pertussis*. In Peru, actual public health programs indicate that vaccination against *B. pertussis* must be mandatory and generalized; besides all detected cases must be reported. The objective of this study was to determine the prevalence of *B. pertussis* among children under five years of age with a presumptive diagnosis of whooping cough in Cajamarca, a region located in northern Peru.

**Materials and Methods:** The population of this cross-sectional study were children under 5 years old hospitalized as presumptive cases of pertussis during December 2017 to December 2018. The nasopharyngeal samples were analyzed by real-time PCR for the detection of *B. pertussis*.

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Results: *B. pertussis* was identified as PCR+ in 42.3% of our sample (33/78). The clinical presentation that was observed most frequently includes paroxysmal coughing (97%), difficulty breathing (69.7%), cyanosis (72.7%) and post-tussive emesis (60.6%). Additionally, pneumonia was the most observed complication (33.3%). Four of the patients with PCR+ for *B. pertussis* presented only lymphocytosis, five only leukocytosis, two patients with decreased leukocytosis and lymphocytes and only one patient with leukopenia and relative lymphocytosis. There was a percentage of 84.8% of unvaccinated children in the PCR+ group. Finally, the mother was the most frequent symptom carrier (18.2%).

Conclusion: In conclusion, in the studied population there is a high rate of PCR+ cases for *B. pertussis*. Laboratory values may show leukopenia or lymphopenia in patients with pertussis. It is necessary to use appropriate laboratory diagnostic tests in all infants with respiratory symptoms for *B. pertussis*. Since, the clinical diagnosis overestimates the diagnosis of pertussis.

Keywords: *Bordetella pertussis*; Whooping cough; Real-time polymerase chain reaction; Peru

INTRODUCTION

Whooping cough also known as pertussis is a major cause of childhood morbidity and mortality worldwide, an estimated 24 million cases and 160,000 pertussis-related deaths occur every year in children under 5 years old (1, 2). Pertussis is an infectious disease caused by the Gram-negative bacterium *Bordetella pertussis*. The infection is characterized for causing an acute affection of the human respiratory tract, viewed as a combination of coughing fits followed by an inspiratory stridor commonly known as the “whoop” (3-5).

Despite widespread vaccination against this pathogen, pertussis has not been eradicated. On the contrary, an alarming resurgence of reported cases has been observed worldwide during the last two decades. Thus, whooping cough remains a persistent global public health problem due to its highly contagiousness, by direct contact through humans or by inhalation, which affects developed countries but mainly low-income nations, and in which the population of children under 5 years old is the most at risk (6-8).

Particularly, in Peru the incidence of *B. pertussis* has experienced a dramatic raise, in 2018 the incidence of the infection per 100,000 inhabitants was 2.5 and it was five times higher than the incidence of 2015 (9). Peruvian vaccination calendar establishes that the immunization must be administered in 4 doses at 2, 4, 6 and 18 months old (10). Unfortunately, this protocol leaves a vulnerability window for newborns and infants in which high morbidity and mortality rates are observed (11-13). Moreover, this especially exposed group is usually the most misdiagnosed, due to their nonspecific clinical presentation of the illness (14, 15).

In this study, the objective was to establish the prevalence of *B. pertussis* in this group of children under 5 years old hospitalized under clinical suspicion of pertussis through molecular diagnosis by polymerase chain reaction (PCR).

MATERIALS AND METHODS

Patients. A cross-sectional study was conducted in Cajamarca, Peru. This region is located in northern Peru at 8,900 feet above sea level in the Andes Mountains with an estimated population of 226,031 inhabitants and has been one of the most affected regions of Peru by *B. pertussis* (9).

A total of 78 children patients under 5 years of age were hospitalized with the presumptive diagnosis of whooping cough in the Hospital Docente Regional de Cajamarca, and they were consecutively studied from December 2017 to December 2018 for the presence of *B. pertussis* by PCR.

Cases with a probable diagnosis of whooping cough are considered to be those who met the criteria and recommendations for case definition of the CDC (Center for Disease Control and Prevention) in Pertussis Surveillance and Reporting (16).

The laboratory data were used to determine the variations in the counting of leucocytes and lymphocytes. The reference values for each age group were obtained from Torrent M and Badell I. (2012) (17).

Ethics statement. This study was approved by the Research Ethics Board of the Hospital Docente Regional de Cajamarca, Peru. The patients were enrolled the samples obtained within the framework of the epidemiological surveillance system, so no written informed consent was required.

Samples. Nasopharyngeal samples were obtained
by experienced laboratory personnel on the first day of hospitalization. One calcium alginate swab (USA) was inserted into each nostril parallel to the palate. The swabs were placed into the same tube containing 2 ml of transport solution (minimal essential medium with 2% fetal bovine serum, amphotericin B 20 µg/ml, neomycin 40 µg/ml).

**DNA extraction.** DNA was extracted from a volume of 200 µL of each sample using a commercial kit (High Pure Template Preparation Kit, Roche Applied Science, Germany) according to the manufacturer’s instructions.

**Real-time PCR for the analysis of *B. pertussis.*** Real-time PCR assay was used for detection *B. pertussis* using the primers and TaqMan probe described by Kosters et al. (18). PCR was performed using a BHQ quencher probe at 100 µM and 50 µM of primers in a final volume of 20 µL and five microliters of the extracted DNA were combined with 15 µL of the master mix. The thermal cycling program was set at 95°C for 10 seconds, 60 cycles of 5 seconds at 95°C, 5 seconds at 57°C and 30 seconds at 72°C; and final extension. All cycles were performed in Light Cycler® 2.0. Instrument and data was analyzed with the LightCycler® Software 4.1 (Roche Diagnostic, Deutschland-Mannheim, Germany).

**Statistical analysis.** Quantitative variables were described as frequencies and percentages for each group. The statistical analysis was performed using the MiniTab 18 and the OriginPro v10 softwares.

**RESULTS**

In our study, 61.5% of patients were infants under 3 months old and this age group is the most predominant of patients positive by *B. pertussis* with 60.6% (20/33) cases. Mothers were the most common family members that were PCR + for *B. pertussis* (Table 1).

The molecular diagnostics using PCR was performed to contrast the presumptive diagnosis of pertussis infection PCR positive and PCR negative results were compared by age group. The most common symptoms presented were paroxysmal cough (94.9%), difficulty breathing (76.9%), cyanosis (60.3%) and post-tussive emesis (51.3%). The only symptoms that presented a significant difference between the PCR+ and PCR- patients were the fever which is most prevalent in PCR- cases (*p < 0.02*), and the cyanosis which was observed with more frequency in PCR+ patients (*p < 0.064*). Furthermore, clinical symptoms were compared by age groups showing that paroxysmal coughing was the most common symptom across all ages (Table 2). The cor-

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**Table 1.** Demographics of patients with whooping cough syndrome and *Bordetella pertussis.*

| Characteristics           | Total of patients n=78 (%) | Patients positive for *B. pertussis* n=33 (%) | Odds ratio (OR) | p-value |
|---------------------------|---------------------------|-----------------------------------------------|-----------------|---------|
| Age                       |                           |                                               |                 |         |
| <3 months                 | 48 (61.5)                 | 20 (60.6)                                     | 0.962           | 0.423   |
| 3-6 months                | 12 (15.4)                 | 5 (15.1)                                      | 0.982           | 0.644   |
| 6-12 months               | 7 (8.9)                   | 2 (6.1)                                       | 0.654           | 0.723   |
| 1-5 years                 | 11 (14.1)                 | 6 (18.2)                                      | 1.354           | 1.000   |
| Gender                    |                           |                                               |                 |         |
| Male                      | 38 (48.7)                 | 15 (45.4)                                     | 0.877           | 0.836   |
| Female                    | 40 (51.3)                 | 18 (54.5)                                     | 1.140           | 0.836   |
| Household contacts        |                           |                                               |                 |         |
| Mother                    | 18 (23.1)                 | 6 (18.2)                                      | 0.741           | 0.624   |
| Father                    | 5 (6.4)                   | 0 (0.0)                                       | 0.000           | 0.319   |
| Siblings < 7 years old    | 11 (14.1)                 | 2 (6.1)                                       | 0.393           | 0.338   |
| Siblings 7 – 10 years old | 9 (11.5)                  | 3 (9.1)                                       | 0.767           | 1.000   |
| Siblings > 10 years old   | 7 (8.9)                   | 3 (9.1)                                       | 1.014           | 1.000   |
| Uncles/ aunts             | 3 (3.8)                   | 1 (3.0)                                       | 0.781           | 1.000   |
| Others                    | 14 (17.9)                 | 8 (24.2)                                      | 1.463           | 0.446   |
| Does not refer data       | 28 (35.9)                 | 14 (42.4)                                     | 1.316           | 0.528   |
relation analysis of the signs and clinical symptoms of the presumptive cases and confirmed by PCR + (total and separated by age) is shown in Fig. 1. In this analysis matrix it is shown that is impossible to discriminate signs and symptoms for a negative or positive diagnosis.

Complications during hospitalization was also registered, pneumonia was the most frequent outcome in 30.8% of the population and 33.3% of the patients PCR+ for *B. pertussis* (Table 2). In addition, 70.5% of patients (55/78) were treated with antibiotics (e.g., macrolides such as azithromycin, ampicillin among the most used), and 69.7% (23/33) of patients PCR+ for *B. pertussis* received antibiotic on day 1 of the hospitalization (Table 3).

Patients PCR + for *B. pertussis* more frequently presented only Leukocytosis (5 cases) (Table 4).

In this study, a large number of children have not been vaccinated, and this occurred both in cases of PCRs- and PCRs- with frequencies of 84.8% (28/33) and 84.4% (38/45), respectively. In the cases of PCRs+ for *B. pertussis*, there was a vaccination coverage of 9.1% for the first dose and 3% for the second and third doses (Table 5).

This research allows studying the seasonal incidence or evolution of frequencies throughout the year. On one hand, Spring and Summer were the seasons with the majority of reported cases. On the other hand, Autumn was the season were most PCR- patients were registered.

**DISCUSSION**

In recent years, a resurgence of *B. pertussis* infections has been observed, various studies assessed in Latin America have reported this resurgence of

| Table 3. Use of antibiotics among total children and those with PCR confirmed *Bordetella pertussis*. |
|-----------------------------------------------|
| | Use of antibiotics | Yes | No |
| | | n (%) | n (%) |
| < 3 months (n=48) | 32 (66.7) | 16 (33.3) |
| 3-6 months (n=12) | 32 (66.7) | 1 (8.3) |
| 6-12 months(n=7) | 5 (71.4) | 2 (28.6) |
| 1-5 years (n=11) | 7 (63.6) | 4 (36.4) |
| Total (n=78) | 55 (70.5) | 23 (29.5) |
| < 3 months (n=20) | 12 (60.0) | 8 (40.0) |
| 3-6 months (n=5) | 5 (100.0) | 0 |
| 6-12 months(n=2) | 2 (100.0) | 0 |
| 1-5 years (n=6) | 4 (66.7) | 2 (33.3) |
| PCR+ (n=33) | 23 (69.7) | 10 (30.3) |

**Table 2. Clinical symptoms among patients with whooping cough syndrome and *Bordetella pertussis***

| Clinical symptoms | Total of patients (n=78) | PCR- (n=45) | PCR+ (n=33) | Patients positive for *B. pertussis* (n=33) |
|-------------------|-------------------------|------------|------------|---------------------------------|
| | | <3 months | 3-6 months | 6-12 months | 1-5 years |
| | | n (%) | n (%) | n (%) | n (%) |
| | Paroxysmal cough | 74 (94.9) | 42 (93.3) | 32 (97.0) | 18 (54.5) | 5 (15.1) | 2 (6.1) | 6 (18.1) |
| | Difficulty breathing | 60 (76.9) | 37 (82.2) | 23 (69.7) | 13 (39.4) | 3 (9.1) | 2 (6.1) | 2 (6.1) |
| | Cyanosis | 47 (60.3) | 23 (51.1) | 24 (72.7) | 15 (45.5) | 3 (9.1) | 1 (3.0) | 5 (15.1) |
| | Post-tussive emesis | 40 (51.3) | 20 (44.4) | 20 (60.6) | 14 (42.4) | 4 (12.1) | 1 (3.0) | 1 (3.0) |
| | Stridor | 38 (48.7) | 26 (57.8) | 12 (36.4) | 6 (18.2) | 1 (3.0) | 2 (6.1) | 3 (9.1) |
| | Breastfeeding difficulties | 33 (42.3) | 22 (48.9) | 11 (33.3) | 9 (27.3) | 0 (0.0) | 0 (0.0) | 2 (6.1) |
| | Fever | 31 (39.7) | 23 (51.1) | 8 (24.2) | 5 (15.1) | 0 (0.0) | 1 (3.0) | 2 (6.1) |
| | Ruddiness | 26 (33.3) | 15 (33.3) | 11 (33.3) | 8 (24.2) | 1 (3.0) | 1 (3.0) | 1 (3.0) |
| | Apnea | 12 (15.4) | 5 (11.1) | 7 (21.2) | 5 (15.1) | 0 (0.0) | 1 (3.0) | 1 (3.0) |
| | Diarrhea | 9 (11.5) | 7 (15.6) | 2 (6.1) | 0 (0.0) | 2 (6.1) | 0 (0.0) | 0 (0.0) |
| | Complications | | | | | |
| | Pneumonia | 24 (30.8) | 13 (28.9) | 11 (33.3) | 4 (12.1) | 0 (0.0) | 1 (3.0) | 1 (3.0) |
| | Acute bronquial obstructive syndrome | 17 (21.8) | 11 (24.4) | 6 (18.2) | 4 (12.1) | 1 (3.0) | 0 (0.0) | 1 (3.0) |
| | Atelectasis | 2 (2.6) | 2 (4.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | Convulsions | 3 (3.8) | 0 (0.0) | 3 (9.1) | 2 (6.1) | 0 (0.0) | 0 (0.0) | 1 (3.0) |
| | Deshidratation | 4 (5.1) | 2 (4.4) | 2 (6.1) | 0 (0.0) | 1 (3.0) | 1 (3.0) | 0 (0.0) |
| | Desnutation | 1 (1.3) | 1 (2.2) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
**Fig. 1.** Correlation analysis of clinical signs and symptoms used to establish the presumptive clinical diagnosis of whooping cough (Total) and the molecular diagnosis of *Bordetella pertussis* (PCR- and PCR+). The groups of <3, 3-6, 6-12 months and 1-5 years correspond to the PCR+ cases. The scatter plots show the pairwise distribution of the data. The ellipse shows the confidence level at 95%. *r* is the Pearson correlation coefficient. *p* is the significant difference value for a 2-tailed t-test.

*B. pertussis* cases (19-21). It is described that infants younger than 6 months old represented up to 70-75% of pertussis cases, and this population also presented the highest morbidity and mortality rates (19-21). A similar scenario was reported in Peru by 3 studies in 2015, and a more recent study in 2017, in which infants younger than 3 months old were the most affected population (22-25). Analogously, in our study, most of our patients with *B. pertussis* were infants under 3 months (Table 1).

In the rural setting of Peru, physicians are doubly challenged, since on top of the heterogeneous, nonspecific clinical presentation of the infection in young infants, they need to manage the limited resources which hinders laboratory confirmation (26, 27). This is the case of Cajamarca, a low-resources region, in which a high prevalence of pertussis is observed (25).

We studied 78 children under 5 years old who were hospitalized as probable cases of pertussis and a total of 33 cases (42.31%) had positive samples for *B. pertussis*, being infants under 3 months old the most affected group (Table 1). This proportion of positive samples is higher than a previous study conducted in 2017, where 20.5% of probable cases for pertussis were positive for the bacterium (25). However, variations are expected as the clinical features have shown to be insufficient to establish a diagnosis and it is estimated that without PCR testing, the overall percentage of missed cases would range from 9 to 26% per year in infants under 6-month-old (28).

The typical clinical presentation of the infection is divided in three distinctive stages and does usually last for 6-10 weeks (29). In addition, in infants younger than 3 months, the disease usually has an atypical presentation, with variation of the duration of the dif-
Table 4. Leukocyte count and percentage of lymphocytes in PCR + patients

| B. pertussis | Blood counts       | ≤ 28 days | 29 days – 6 months | 3-5 months | 6-11 months | 1-5 years | Total |
|--------------|--------------------|-----------|--------------------|------------|-------------|-----------|-------|
| Positive     | Leukocytosis       | 0         | 3 (9.0)            | 2 (9.0)    | 1 (3.0)     | 2 (6.0)   | 12 (6.0) |
|               | Lymphocytosis      | 0         | 3 (9.0)            | 2 (9.0)    | 1 (3.0)     | 2 (6.0)   | 12 (6.0) |
| n=33(%)      | Leukocytosis and Lymphopenia | | | | | | |
|              | Leukopenia and Lymphocytosis | | | | | | |
| Total        | 0                  | 8 (24.0)  | 2 (6.0)            | 0          | 2 (6.0)     | 12 (36.0) |

Table 5. Vaccination coverage status among total children and those with PCR confirmed *Bordetella pertussis*.

|                   | 1 dose | 2 doses | 3 doses | None |
|-------------------|--------|---------|---------|------|
| < 2 months (n=35) | --     | --      | --      | 35   |
| 2m - <4 months (n=21) | 5   | 0       | --      | 16   |
| 4m - <12 months (n=11) | 2 | 1       | 1       | 7    |
| 1-5 years (n=11)  | 0      | 1       | 2       | 8    |
| Total n=78 (%)    | 7 (9.0) | 2 (2.6) | 3 (3.8) | 66 (84.6) |
| < 2 months (n=14) | -- | -- | -- | 14 |
| 2m - <4 months (n=9) | 2 | 0 | -- | 4 |
| 4m - <12 months (n=4) | 1 | 0 | 1 | 6 |
| 1-5 years (n=6)  | 0      | 1       | 0       | 5    |
| PCR+ n=33 (%)     | 3 (9.1) | 1 (3.0) | 1 (3.0) | 28 (84.8) |

Different stages and the absence of the most characteristics sign, the “whoop”. Also, other features like lack of fever, gagging, gasping, choking, cyanosis, bradycardia, and vomiting are presented on these group (11, 15). This described clinical presentation matches the findings in our study, since there was a statistical difference between patients PCR+ and PCR- only in two clinical features, fever that is more prevalent on the PCR- group and cyanosis which was more observed in PCR+ group. Additionally, it also has been reported that post-tussive vomiting is common at all ages (11, 15); in our series, it was present in 60.6% (20/33) of patients with *B. pertussis* (Table 2).

It has been demonstrated that a whooping cough alone is not enough to start antibiotics immediately, especially in infants younger than 4 months (30). However, in rural areas where laboratory resources are limited physician usually give macrolides when there is high suspicion of pertussis. In our population, 70.5% (55/78) of patients received antibiotics on day 1 of hospitalization. In addition, 69.7% (23/33) of patients with *B. pertussis* were covered before we report their results as positive, and the other 10 cases were started on antibiotics the same day we sent our results (Table 3).

Leukocytosis with an absolute lymphocytosis, which are more accessible laboratory tools in the rural setting, are usually present at the end of the cattathal stage (14, 15, 26). Since in our series the leukocytosis and lymphocytosis values were adjusted to each age group, in comparison to other studies were cell counts were considered high when compared to fixed values across all ages, we were expecting a lower number of patients PCR+ with these clinical signs (18, 26). However, we found that patients with PCR for *B. pertussis* may present: only lymphocytosis, only leukocytosis, leukocytosis with lymphopenia and also leukopenia with relative lymphocytosis. This shows the diversity of results in relation to the values of leukocytes and lymphocytes in patients with pertussis (Table 4).

The mother has been identified as the most common source of infection in up to 63% of cases, followed by fathers, siblings and other family members (19, 31). In our study, possible household contacts were also tested for the presence of *B. pertussis*, and mothers were the most common infected familiar with a percentage of 18.2% (6/33) (Table 1).

We observed that 73.7% (14/19) of our patients older than 2 months old with *B. pertussis* did not receive
any vaccination at all. In addition, it is well known that the first dose often provides scarce protection (8). Thus, children with incomplete vaccination are prone to be infected by the bacterium as we found 4 cases of pertussis with incomplete immunization.

CONCLUSION

In the studied population there is a high rate of PCR+ cases for B. pertussis. No significant difference was observed between the clinical features of the two groups, PCR+ and PCR – patients (Table 1), which underlines the need to use appropriate laboratory diagnostic tests in all infants with respiratory symptoms for B. pertussis. Laboratory values may show leukopenia or lymphopenia in patients with pertussis. Since, the clinical diagnosis overestimates the pertussis diagnosis.

This study has one limitation, it was conducted in a restricted hospital setting that would not be representative of the whole country and is potentially biased by the selection criteria requiring hospitalization. Nonetheless, our findings may contribute to describe B. pertussis infection in unimmunized population of small infants.

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