Duration of *Bordetella pertussis* Polymerase Chain Reaction Positivity in Confirmed Pertussis Illness

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Pertussis cases have been increasing in the United States for the past 40 years. Polymerase chain reaction (PCR) testing is now the preferred method of diagnosis, but little is known about duration of PCR positivity.

We conducted a pilot study using serial *B pertussis* PCR testing to determine duration of PCR positivity. Kaplan-Meier survival analysis suggested a median duration of 58 days (interquartile range, 40–110 days).

Key words. pertussis; pertussis diagnosis; pertussis infection; pertussis PCR.

**BACKGROUND**

**Case Report**

Baby E was a 38-day-old infant in respiratory failure who was admitted to the pediatric intensive care unit. She was found to be *Bordetella pertussis* polymerase chain reaction (PCR) positive, with direct florescence antigen negative (no culture). After a 6-week, in-patient stay prolonged by frequent cough paroxysms inducing apneas, she improved and was discharged. Within 4 weeks, she returned with coughing paroxysms and a “whoop,” 62 days after original symptom onset and after therapy with erythromycin 40 mg/kg per day for 14 days with clinical improvement. *Bordetella pertussis* PCR was again positive. She was treated with trimethoprim-sulfamethoxazole to cover a possible resistant strain [1]. We were left not knowing whether this condition represented a macrolide-resistant initial infection, a second pertussis infection, or a different infection with persisting pertussis PCR positivity.

Diagnosis of pertussis illness by PCR has increased in recent years, particularly as real-time assays with quick turn-around and high sensitivity and specificity were developed [2–4]. Experts suggested that PCR positivity might exhibit a relatively narrow window of diagnostic opportunity similar to culture (<21 days), perhaps extending slightly due to PCR sensitivity [5, 6]. The actual duration of PCR positivity has not been determined. Our pilot study addresses this knowledge gap through serial testing of patients who were PCR positive.

**MATERIALS AND METHODS**

This research was a prospective cohort study conducted from July 2004 through August 2006 at Primary Children’s Medical Center, a tertiary care children’s hospital in Salt Lake City, Utah, that provides care to children in the Intermountain West. The study was approved by the University of Utah Institutional Review Board and the Intermountain Healthcare privacy board.

Index cases were hospital admissions for pertussis illness in children <18 years with a positive *B pertussis* PCR; all patients met the Centers for Disease Control and Prevention (CDC) laboratory-confirmed definition of *B pertussis* infection [7]. Associated cases were close contacts with at least 7 days of cough illness and no known explanation. Subjects who were enrolled provided nasopharyngeal (NP) samples for PCR testing at baseline and checked weekly for 3 weeks, then monthly or every other month, for 1 year from symptom onset, until samples yielded a negative result.

If the first PCR was positive, or if PCR was negative but initial sampling occurred after 21 days and subjects met the CDC-confirmed case definition, subjects were considered true *B pertussis* cases. If the first PCR was negative and sampling occurred during the first 21 days of illness, irrespective of meeting or not meeting the CDC clinical case definition, or if the first PCR result was negative, sampling occurred after 21 days, but the CDC-confirmed case definition was not met, then *B pertussis* infection was
excluded from further analysis. Only true cases were included in the analysis. Polymerase chain reaction results were reported to participants, and positive results were reported to the participant’s state of residence Department of Health, as required by law. Participants were aware of the legal reporting requirement. The participant’s primary physician prescribed antibiotics as all enrollees met criteria for prophylaxis or treatment. The study protocol did not delay antibiotics. Participants completed a data collection sheet that defined the date of symptom onset, symptom details, treatment, and immunization status.

Nasopharyngeal samples were obtained by NP suctioning for infants (6- or 8-French catheter with inline trap) or a sterile Dacron swab inserted into the posterior nasopharynx for older children and adults. Samples were stored at −70°C for batch analysis.

Polymerase chain reaction testing was completed at ARUP Laboratories (Salt Lake City, UT) using LightCycler technology to identify a 234-basepair (bp) fragment of the IS481-specific primers to detect B pertussis (Roche Molecular Systems, Inc., Branchburg, NJ). A 200-bp fragment of the IS1001 gene of Bordetella parapertussis was also amplified using specific primers (GenBank accession numbers M28220 and X66858). The resulting amplicons were detected by fluorescence using 2 pairs of hybridization probes, with each pair having sequence homology to portions of the IS481/1001 genes and shown to hybridize exclusively, under stringent conditions, to these genes. The hybridization probes were labeled with fluorescein (3 probe end) and LightCycler Red 640-NHS (5’ probe end) to determine the presence of the IS481 and IS1001 genes during PCR, with detection through fluorescence resonance energy transfer (FRET) when both probes hybridize, bringing the fluorescing molecules in close proximity. An internal control was present in all samples, with separate hybridization probes using FRET at 705 nm to control for amplification inhibition. LightCycler color compensation calibration was achieved prior to runs, and controls without template DNA were included [3].

Survival analysis of PCR positivity was undertaken using STATA 12 (StataCorp, College Station, TX). Each PCR result with its linked “days from symptom onset” time point provided a data pair for analysis, by patient. Censoring occurred at the last positive result, favoring a conservative survival estimate, or last sampling time, if the first result was negative, or if there was no follow-up. The primary outcome represents a “temporal history of treated disease” observation and is presented as a Kaplan-Meier survival estimate of median days of PCR positivity with 95% confidence interval.

RESULTS
Fifty-two subjects consented to participate in the study, and 49 subjects completed the study, including 14 index cases and 35 associated cases. Among the associated cases, 18 (51%) were PCR positive; if subjects were limited to the 26 who met the CDC clinical criteria, 69% were PCR positive. Thirty-one PCR-positive subjects and 3 PCR-negative subjects (sampled >21 days after symptom onset [30, 32, and 42 days] and met CDC clinical criteria) were included in the analysis. Pertussis illness was excluded in the remaining 15. Among the 31 positives that could have participated in serial sampling, 7 lived >35 miles away and 2 (29%) participated, whereas 24 lived <35 miles away and 11 (46%) participated. Four were still positive at dropout. From symptom onset, positive samples spanned from 4 to 172 days, and in PCR-positive subjects with serial sampling, conversion to negative occurred between 22 and 207 days. The analysis included 34 patients who provided 50 PCR samples. Of these, 33 had documented treatment with either erythromycin (40 mg/kg per day or 2 g per day, x14 days) or azithromycin (10 mg/kg per day on day 1 then 5 mg/kg per day for 4 days or 500 mg day 1, then 250 mg daily for 4 days). The Kaplan-Meier survival analysis showed a median of 58 days PCR positivity (95% confidence interval, 40–110 days) (see Figure 1). No subjects were positive for B parapertussis.

DISCUSSION
We found that B pertussis PCR results remain positive a median of 58 days after symptom onset, despite subjects completing a course of appropriate antibiotics and
showing improved clinical symptoms. Clinicians and the pertussis research community should consider persistent positivity when interpreting PCR test results in patients with cough illness and suspicion of pertussis. A repeat positive *B* pertussis PCR study later in the course of illness might be expected, and an alternative diagnosis could be entertained. The prolonged presence of *B* pertussis DNA also raises questions in relation to infectivity risk beyond 21 days of illness or 5 days after starting antibiotic treatment.

We are not aware of a similar published study regarding persistence of PCR positivity in *B* pertussis illness. Other experts have suggested a window for PCR testing similar to that reported for culture of *B* pertussis (≤21 days), or possibly extending testing to 28 days. In other studies, researchers suggest the window for PCR testing might be significantly longer [5, 6, 8–10]. We have presented evidence that this testing may be extended to nearly 6 months.

In this pilot study, limitations include small sample size, reflected in the wide 95% confidence interval of our results, and few single subject serial measurements. A challenge that faces researchers at referral centers serving geographically large areas is difficulty for subjects to return for serial measurements. By repeating the study in an ambulatory care setting to increase enrollment and serial measurements, our findings could rapidly be confirm. Ascertainment of true pertussis illness was straightforward for those who were PCR positive, those who were PCR negative within 21 days, or those who were negative and did not meet the CDC clinical case definition if sampled >21 days. Adding the assumption that negative testing >21 days in subjects meeting the CDC clinical case definition were true pertussis illness had little impact on our study (3 subjects added), and in our case this would bias toward a shorter mean days of PCR positivity. Length of antibiotic exposure was unavailable for 3 subjects. Lastly, we were limited by potential recall bias regarding date of symptom onset. The date of onset was carefully explored with subjects in an attempt to reduce recall bias. Nearly all subjects described memorable symptoms associated with the date of onset, and most subjects reported specific dates rather than date ranges or expressing uncertainty in other ways.

**CONCLUSION**

*Bordetella pertussis* PCR may remain positive in 50% of patients for >50 days after the onset of symptoms, despite antibiotic treatment and clinical improvement. Testing for *B* pertussis by PCR when clinical suspicion of infection exists may be useful weeks after symptom onset; respiratory illness testing strategies need to include consideration that persistent positive *B* pertussis PCR results may confuse the diagnosis in nonpertussis infection.

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