



## Diagnosing Pertussis: The Role of Polymerase Chain Reaction

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### Abstract

**Background:** Whereas the diagnosis of classical pertussis has traditionally been based on clinical criteria, increasing numbers of atypical presentations suggest the need for an extensive laboratory-based approach.

**Objectives:** To assess the relative efficacy of clinical and laboratory methods in the diagnosis of *Bordetella pertussis* by patient age and immunization status.

**Methods:** We compared the clinical and laboratory diagnosis of *B. pertussis* in 87 pre-vaccinated, 78 recently vaccinated, and 75 post-vaccinated children with suspected pertussis. Serum and nasopharyngeal swabs were obtained for serology, culture and polymerase chain reaction.

**Results:** PCR and culture identified 41% and 7% of patients with *B. pertussis*, respectively ( $P < 0.001$ ). All positive cultures were PCR-positive. Positive PCR was less common among those recently vaccinated than among those in the pre- ( $P < 0.001$ ) and post-vaccinated groups ( $P < 0.05$ ). Positive culture was more common among those pre-vaccinated than among those recently vaccinated ( $P < 0.01$ ). Positive tests for immunoglobulin M and A were more common among the post-vaccinated than the pre- and recently vaccinated ( $P < 0.001$ ), respectively. Logistic regression analyses revealed that clinical criteria have no significant association with infection in recently and post-vaccinated children. Among the pre-vaccinated children, whoop and cough duration were associated with a positive PCR (odds ratio 7.66 and 0.5,  $P < 0.001$ ). Seventy-six percent of pre-vaccinated, 39% of recently vaccinated and 40% of post-vaccinated children with positive PCR did not meet the U.S. Centers for Disease Control diagnostic criteria for *B. pertussis*.

**Conclusions:** PCR is a useful tool for pertussis diagnosis, particularly in pre-vaccinated infants. The yield of culture and serology is limited, especially among pre- and recently vaccinated children. In pre-vaccinated infants with whoop and less than 2 weeks of cough, PCR testing should be implemented promptly.

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While widespread vaccination against *Bordetella pertussis* initially resulted in a decline of disease over the last decade, there has been a dramatic increase in the incidence of disease among both vaccinated and unvaccinated individuals [1–3]. In the United States close to 8,000 cases were reported in 1996, the highest number reported since 1967 [4]. The resurgence of pertussis is not surprising given that the effects of the vaccination wane after 5–10 years, rendering the vaccinated host vulnerable to infection [5]. Indeed, in many countries there have been increasing reports of pertussis among vaccinated individuals among whom the clinical presentation is more protracted or atypical [1,6,7]. We recently tested 46 fully vaccinated children in daycare centers who had been exposed to a fatal case of pertussis and found that although 5 (11%) of the children were colonized with *Bordetella pertussis* (positive polymerase chain reaction), only 2 of these children had the typical course of pertussis infection [8]. Recent incidence data from the U.S. indicate that there has also been a shift in the age distribution of cases, with 29% aged 10–19 years [9]. This is significant, since these individuals and adults typically serve as a reservoir for transmission to pre-vaccinated infants among whom the disease tends to be more pernicious [10–15]. In a recent study exploring the epidemiology of reported cases of pertussis, family members were found to be the primary source of pertussis among those infants with an identifiable source; in particular, mothers were the primary source of infection in infants up to 3 months old [16].

In the current study, we sought to study the variance in the clinical and laboratory manifestation of pertussis by age and immunization status, and to assess the relative efficacy of clinical and laboratory standards in the diagnosis of the disease by immunization status.

### Subjects and Methods

#### Subjects

Our sample consisted of 306 individuals with an upper respiratory infection suspicious for pertussis, who were referred by their physician to a major medical center in northern Israel between

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\* This research was conducted at Bnai Zion Medical Center and Carmel Medical Center, both in Haifa, Israel

PCR = polymerase chain reaction

December 1999 and January 2001. Patients diagnosed with otitis media, bronchiolitis or pneumonia were excluded from the study. Also excluded were those without complete vaccination data or appropriate vaccination for age (at least 5 years old with fewer than four doses, or age over 1 year with fewer than three doses). All patients were under 18 years old. The study was approved by the medical center's Helsinki Committee.

#### Data collection and laboratory methods

Data on clinical demographics, symptomatology and possible exposure were collected by means of a questionnaire. The sample was then divided according to immunization status as follows: Group 1: pre-vaccinated infants  $\leq 1$  year old who received 0–1 vaccine doses of DTP (diphtheria, tetanus, pertussis) ( $n=87$ ); Group 2: recently vaccinated children under 5 years old who received 3–4 doses, as well as those  $\leq 1$  year who received 2 doses ( $n=78$ ); Group 3: post-vaccinated individuals older than 5 years who received 4 doses ( $n=75$ ). When vaccinated, all children were immunized with the Pasteur vaccine containing one immunization dose of purified diphtheria toxoid, 1 ID of purified tetanus toxoid, and  $>4$  IU of *B. pertussis*, i.e., whole-cell pertussis vaccine

#### PCR analysis

Two nasopharyngeal Dacron swab/aspirate specimens (Medical wire, MEDECO, UK) were collected for culture and PCR testing. The specimens for PCR testing were stored in 1–2 ml of sterile physiologic saline solution and promptly transferred to the laboratory where they were kept at  $-70^{\circ}\text{C}$  until processing. Primers specific for the repeated insertion sequences of *B. pertussis* genome were used in a semi-nested PCR assay as previously described [17]. Potential inhibitory activity was monitored by amplifying DNA from negative samples together with minimal quantities of *B. pertussis* DNA obtained from cultures. The laboratory adhered to standard PCR precautions. The analytical sensitivity of the assay assessed by serial dilution of a determined DNA copy number showed the assay to be sensitive to four bacteria. The diagnostic specificity was assessed by testing 100 nasopharyngeal swabs collected from asymptomatic children, ages 1–4 years, in a daycare setting, and was found to be 96% specific (provided courtesy of Prof. R. Dagan, Beer Sheva, Israel).

#### Culture

The culture specimen was plated on charcoal agar plates (HyLabs, Rehovot, Israel) and incubated at  $37^{\circ}\text{C}$  for 14 days. Colonies suspected to be *B. pertussis* underwent standard biochemical reactions and the confirmation of the organism was instituted by agglutination with appropriate antiserum.

#### Serology

Immunoglobulin A, M and G antibodies to *B. pertussis* were measured by an enzyme-linked immunosorbent assay using whole-cell lysate

antigens (Pan Bio, Australia) [18]. Antibodies were expressed as ELISA units according to the manufacturer's guidelines.

#### Data analysis

Univariate and multivariate analyses were performed to examine the clinical and laboratory manifestation of *B. pertussis* and to assess the sensitivity and specificity of each diagnostic parameter. Logistic regression analyses (controlling for gender, age and number of doses) for both the full sample and each of the immunization-based subgroups were conducted to evaluate the clinical and laboratory correlates of a positive PCR. All reported *P* values were considered significant at  $<0.05$ .

## Results

#### Sample composition

Of the 240 subjects, a nasopharyngeal specimen was collected for culture from 219, PCR was performed for 238, and serum specimens were collected from 214 and 231 for IgA and IgM testing respectively. Among those for whom gender data were available, there was an equal number of males and females. The mean age was 4.08 years (SD 5.05), range 0.05–17.7 years. In the overall sample 93.5% did not receive any antibiotic treatment prior to specimen collection.

#### Laboratory analysis

As shown in the upper part of Table 1, 98 of 238 patients (41.2%) had a positive PCR, 16/219 (7.3%) had a positive culture, 49/214 (22.9%) had positive IgA and 71/231 (30.7%) had positive IgM. All culture-positive patients were also PCR-positive. PCR

**Table 1.** Clinical and laboratory symptomatology by group (% positive)

Method	Population (n=240)	Pre-vaccinated (n=87)	Recently vaccinated (n=78)	Post-vaccinated (n=75)
PCR*	41.18	54.02	24.36	43.84
Culture**	7.31	15.48	0	5.17
IgM#	30.74	4.82	21.05	70.83
IgA##	22.90	2.70	13.33	56.92
Cough, <1 week	22.61	39.02	17.11	9.72
Cough, 1–2 weeks	26.09	26.83	32.89	18.06
Cough, 2–3 week	18.70	14.63	17.11	25.00
Cough, >3 week	32.61	19.51	32.89	47.22
Fever	26.72	21.95	38.96	19.18
Cyanosis	21.55	43.9	11.69	6.85
Paroxysmal cough	80.17	86.59	80.52	72.60
Whooping	38.79	48.78	37.66	28.77
Apnea	12.93	25.61	7.79	4.11
Post-tussive vomiting	41.10	40.24	45.45	41.46

\* Pre > recent ( $P < 0.001$ )

\*\* Pre > recent ( $P < 0.01$ )

# Post > pre ( $P < 0.001$ ), post > recent ( $P < 0.001$ ); recent > pre ( $P < 0.05$ )

## Post > pre ( $P < 0.001$ ); post > recent ( $P < 0.001$ ).

ID = immunization dose

ELISA = enzyme-linked immunosorbent assay

Ig = immunoglobulin

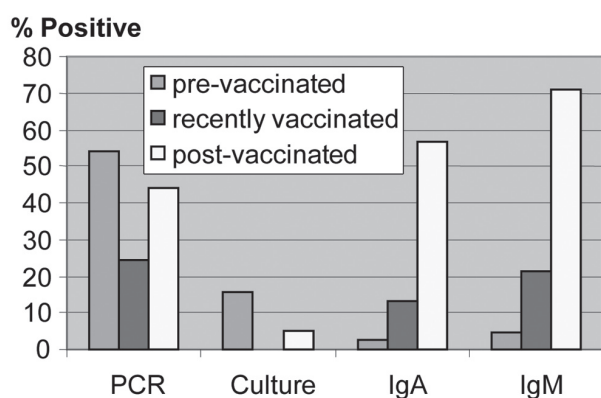


Figure 1. Laboratory confirmation of pertussis by vaccination status

was significantly lower among those recently vaccinated (19/78, 24%) than among those in the pre-vaccinated group (47/87 or 54%; Scheffe = -0.30,  $P < 0.001$ ) [Figure 1]. Similarly, culture isolation was more common among the pre-vaccinated group than among patients recently vaccinated (13/84 or 15% vs. 0/77 or 0%; Scheffe = -0.155,  $P < 0.01$ ). Positive IgA for pertussis was more common among those post-vaccinated (37/65, or 56.9%) as compared with those pre- (2/74 or 2.7%; Scheffe = -0.54,  $P < 0.001$ ) or recently vaccinated (10/75 or 13.3%; Scheffe = -0.44,  $P < 0.001$ ). Positive IgM was significantly more common in the post-vaccinated group than in either the pre-vaccinated or recently vaccinated groups (51/72 or 70.8% versus 4/83 or 4.8% Scheffe = -0.66,  $P < 0.001$ , and 16/76 or 21.1%, Scheffe = -0.50,  $P < 0.001$  respectively), and was significantly more common in the recently vaccinated group than in the pre-vaccinated group (Scheffe = -0.16,  $P < 0.05$ ).

### Clinical analysis

Selected clinical symptomatology is presented in the lower part of Table 1. To gain a better understanding of how the predictive value of the various clinical predictors may vary by immunization status, a logistic analysis using PCR as the criterion for infection was performed. Clinical characteristics were significant predictors of pertussis infection in the pre-vaccinated group only. Among those in this group, whoop was a strong predictor of infection (odds ratio 7.66,  $P < 0.001$ ), indicating that even when controlling for all other clinical symptoms, those patients reporting whoop were more than seven times more likely to be infected with pertussis than those not reporting this symptom. Cough duration was also a statistically significant clinical marker of infection in the pre-vaccinated group (OR 0.52,  $P < 0.05$ ), indicating that for each additional week of cough duration the likelihood of infection declines by over 50%. A decomposition analysis indicated that sensitivity is greatest with cough duration of 1 week or less (41.3% with a positive PCR), declining as

duration increases and eventually leveling out at 13.04% with a positive PCR at 2–3 weeks.

### Discussion

Our findings suggest that there is substantial variation in both the sensitivity of laboratory diagnostic methods and the clinical manifestations of pertussis when stratified according to vaccination status.

#### Laboratory diagnostic methods

As noted above, the variance across groups was statistically significant with regard to all three laboratory markers. Consistent with previous knowledge that isolation rates are negatively correlated with increasing age and the number of pertussis vaccination doses received by the patient [19], the isolation of *B. pertussis* was most successful in the pre-vaccinated group. In this group the serology yield was low, possibly because the production of measurable antibodies may be delayed in infants [19]. In contrast to the infants, positive serology was frequent in the post-vaccinated group in whom diagnosis is frequently delayed and often culture-negative.

Regardless, neither of these two laboratory markers approached the high sensitivity levels achieved by PCR. Our results support the use of PCR as a useful tool for pertussis diagnosis for all three vaccination groups. Among pre-vaccinated infants, PCR testing may offer the most effective means of diagnosing pertussis due to the poor sensitivity of serology and the low yield of culture among individuals in this group. Among recently and post-vaccinated individuals, in whom diagnosis is frequently delayed and the recovery of the organism rare, PCR may be equally advantageous. In the current study, PCR identified the majority of cases in the pre-vaccinated group, but it also played a significant role in the recently vaccinated and post-vaccinated groups. Given the poor sensitivities of serology and culture in the pre-vaccinated group, PCR testing should be used to complement these markers. This recommendation is consistent with the revised pertussis-related diagnostic criteria of the U.S. Centers for Disease Control. The CDC now recommends that serologic test results no longer be relied upon for pertussis case confirmation [14]. Despite its low sensitivity, culture must be obtained in all patients to determine antimicrobial resistance patterns and the molecular typing of the organism [14].

#### Clinical symptomatology

Similar variation across groups was found with respect to clinical symptomatology. Shorter cough duration and a higher prevalence of whooping were found among pre-vaccinated infants. This is consistent with the findings of Halperin and associates [20] who found whooping to be less frequent among older children. Similarly, atypical symptomatology (defined as a lower prevalence of whooping and paroxysmal cough) was relatively more prevalent among recently vaccinated and post-vaccinated children. Consistent with the conclusions reached by Khetsuriani et al. [21], the implication of this finding is that strict reliance on clinical criteria may

OR = odds ratio

CDC = Centers for Disease Control

result in a substantial delay in diagnosis among recently and post-vaccinated individuals, thus delaying treatment and infection control.

Whereas in the pre-vaccine era when classical pertussis prevailed and its diagnosis was frequently based on clinical symptomatology, today, with widespread vaccination and atypical manifestations, clinicians need to pursue laboratory testing to establish the diagnosis. Using PCR as our criterion measure, none of the clinical criteria (e.g., paroxysmal cough, whoop) was found to have a significant association with infection (positive PCR). For pre-immunized infants the findings are more complex. While both whoop and cough duration were found to be robust predictors of infection, prolonged cough duration was found to significantly *reduce* the odds of infection among pre-vaccinated infants. These findings suggest that "classic" clinical manifestations of pertussis may not necessarily be evident, and that clinicians recognize that clinical symptomatology is influenced by patient age and immunization status. The findings also suggest that for pre-vaccinated infants with whoop and less than 2 weeks of cough, PCR should be performed promptly. This is consistent with the findings of Muller et al. [19] who suggest that PCR plays a role in the diagnosis of pertussis in individuals of all ages and regardless of treatment status, particularly when cough duration is 2 weeks or less. Such prompt diagnosis may be important in reducing the household spread of the disease and may facilitate early medical intervention.

## Conclusion

Our results raise questions regarding the possible need to re-examine the predictive value of existing CDC diagnostic criteria for specific population subgroups, and to possibly identify new sets of criteria specific to particular subgroups based on age and vaccination status. In the meantime, as noted above, our results suggest that there may be substantial benefit in performing PCR on all pre-vaccinated infants presenting with whoop and cough symptoms, irrespective of duration of symptoms. Furthermore, from an epidemiologic and research perspective, our findings suggest the need to collect pertussis-related data of family members with cough. Such data are critical to ascertain the degree to which the steady increase in the incidence of pertussis noted earlier may be attributable to increased transmission from adults and adolescents to young children.

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## References

- Christie CD, Marx ML, Marchant CD, Reising SF. The 1993 epidemic of pertussis in Cincinnati. Resurgence of disease in a highly immunized population of children. *N Engl J Med* 1994;331:16–21.
- Black S. Epidemiology of pertussis. *Pediatr Infect Dis J* 1997;16 (Suppl 4):S85–9.
- Cherry JD, Heininger U. Pertussis and other Bordetella infections. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Diseases*. 4th edn. Philadelphia: Lippincott Williams & Wilkins, 1998:1423–40.
- Global Programme on Vaccines: State of the World's Vaccines and Immunization. Geneva: World Health Organization and UNICEF, 1996.
- Jenkinson D. Duration of effectiveness of pertussis vaccine: evidence from a 10-year community study. *Br Med J* 1988;296: 612–14.
- Cherry JD. Epidemiological, clinical, and laboratory aspects of pertussis in adults. *Clin Infect Dis* 1999;28(Suppl 2):S112–17.
- Yaari E, Yafe-Zimmerman Y, Schwartz SB, et al. Clinical manifestations of *Bordetella pertussis* infection in immunized children and young adults. *Chest* 1999;115:1254–8.
- Srugo I, Benilevi D, Madeb R, et al. Pertussis infection in fully vaccinated children in day-care centers, Israel. *Emerg Infect Dis* 2000;6:526–9.
- Centers for Disease Control and Prevention. Pertussis – United States, 1997–2000. *MMWR* 2002;51:73–6.
- Christie CD, Baltimore RS. Pertussis in neonates. *Am J Dis Child* 1989;133:199–202.
- Heininger U, Stehr K, Cherry JD. Serious pertussis overlooked in infants. *Eur J Pediatr* 1992;151:342–3.
- Smith C, Vyas H. Early infantile pertussis: increasingly prevalent and potentially fatal. *Eur J Pediatr* 2000;159:898–900.
- Nelson JD. The changing epidemiology of pertussis in young infants: the role of adults as reservoirs of infection. *Am J Dis Child* 1978;132:371–3.
- 14 [Http://www.cdc.gov/health/diseases/pertussis](http://www.cdc.gov/health/diseases/pertussis). Chapter 6, pp. 67–84
- Hewlett E. Bordetella species. In: Mandell G, Bennett J, Dolin R, eds. *Principles and Practice of Infectious Diseases*. 5th edn. New York: Churchill Livingstone, 2000:2414–19.
- Bisgard K, Pascual FB, Ehresmann K, et al. Infant pertussis: who was the source? *Pediatr Infect Dis J* 2004;23(11):985–9.
- Lichinghagen R, Diedrich-Glaubitz R, von Horsten B. Identification of *Bordetella pertussis* in nasopharyngeal swabs using a polymerase chain reaction: evaluation of detection methods. *Eur J Clin Chem Biochem* 1994;32:161–7.
- He Q, Mertsola J, Soini H, Viljanen MK. Comparison of polymerase chain reaction with culture and enzyme immunoassay for diagnosis of pertussis. *J Clin Microbiol* 1993;31:642–5.
- Muller FM, Hoppe JE, Wirsing von Konig CH. Laboratory diagnosis of pertussis: state of the art in 1997. *J Clin Microbiol* 1997;35 (10):2435–43.
- Halperin SA, Bortolussi R, MacLean D, Chisholm N. Persistence of pertussis in an immunized population: results of the Nova Scotia enhanced pertussis surveillance program. *J Pediatr* 1989;115:686–93.
- Khetsuriani N, Bisgard K, Prevots DR, et al. Pertussis outbreak in an elementary school with high vaccination coverage. *Pediatr Infect Dis J* 2001;20:1108–12.

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