# Progress in the Diagnosis, Prevention, and Treatment of Pertussis

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Pertussis ("whooping cough"), caused by the gram-negative pleomorphic bacillus *Bordetella pertussis*, is a highly contagious, potentially life-threatening respiratory tract illness that has re-emerged worldwide as a cause of substantial morbidity and mortality in infants, children, and adolescents, even in countries with high vaccination rates. Waning immunity after immunization during childhood has been associated with a growing pool of susceptible adolescents and adults who are capable of transmitting pertussis to vulnerable unvaccinated or incompletely vaccinated infants. The use of acellular pertussis vaccine boosters in adolescents has been proposed and is likely to be recommended. Active immunization and improved methods for early diagnosis are key in the management of pertussis, and represent the most rapidly evolving aspects of this disease.

## Introduction

The introduction and widespread use of whole cell pertussis vaccines combined with diphtheria and tetanus toxoids for routine childhood immunization in the United States in the 1940s led to a significant and sustained decrease in the incidence of pertussis. The high rates of adverse reactions (local reactions, fever, and systemic symptoms) associated with this vaccine prompted the development of a less reactogenic second generation of acellular pertussis vaccines (DTaP). The efficacy of DTaP is comparable to the whole cell pertussis vaccine when used as part of the primary three-dose series for infants, although the duration of protection after immunization has not definitively been established [1]. DTaP is now used in the United States and other developed countries. The schedule in the United States consists of vaccination at 2, 4, 6, and 15 to 18 months of age with a booster dose at 4 to 6 years of age [2]. Similar schedules are recommended in other areas of the world, with some countries using an accelerated three-dose primary infant series at 2, 3, and 4 months. Pertussis vaccines are not licensed for use in children over the age of 7 years in the United States.

Despite infant immunization rates against pertussis exceeding 80% in many countries, there has been a cyclical rise in disease incidence since the early 1980s, with peaks occurring every 3 to 4 years (Fig. 1). Adolescents and adults in whom vaccine-acquired immunity may have waned, and who often have mild or atypical illness, are now recognized as an important reservoir of pertussis. During 2002, reports of pertussis in children associated with adolescent or adult cases were published from the United States [3•,4], Canada [5•,6], Europe [7,8], Australia [9,10], and China [11]. The most disturbing trend was observed in British Columbia, Canada, where the incidence of pertussis among preteens and adolescents surpassed that of all other age groups, while a decreasing incidence was noted among infants and preschool children. This trend suggests that while immunization is quite effective in the very young, the issue of waning immunity during the second decade of life needs to be addressed [5•]. Emergence of vaccine-resistant strains, another potential cause for resurgence of pertussis, is under investigation using molecular typing methods [12,13].

As many as 90% of nonimmune household contacts acquire pertussis from an index case, the most vulnerable being under- or unimmunized infants. Epidemiologic data for the period 1997 through 2000 in the United States reveal a crude average annual incidence of 2.7 per 100,000 population with increasing rates each year (Fig. 1) [3•]. Average annual incidence rates were highest in infants less than 1 year of age (55.5/100,000 or 29% of all cases) (Fig. 2). The proportion of patients hospitalized or who had complications of pertussis illness were highest in infants less than 6 months of age (63% hospitalized, 12% pneumonia, 1% seizures, 0.2% encephalopathy, 0.8% mortality) and decreased with increasing age. In the year 2000, all 17 pertussis-related deaths reported to the Centers for Disease Control and Prevention (CDC) occurred in US born infants who contracted their illness at less than 4 months of age [14•]. As of November 9, 2002, 6531 cases of pertussis had been reported to the CDC, representing a 37% increase over the number of cases reported last year to this date. These data are echoed in the state of Texas, where as of August 30, 2002, the number of reported cases (725) had exceeded the total reported in 2001 (615) and included four deaths, all in infants under 3 months of age [15].

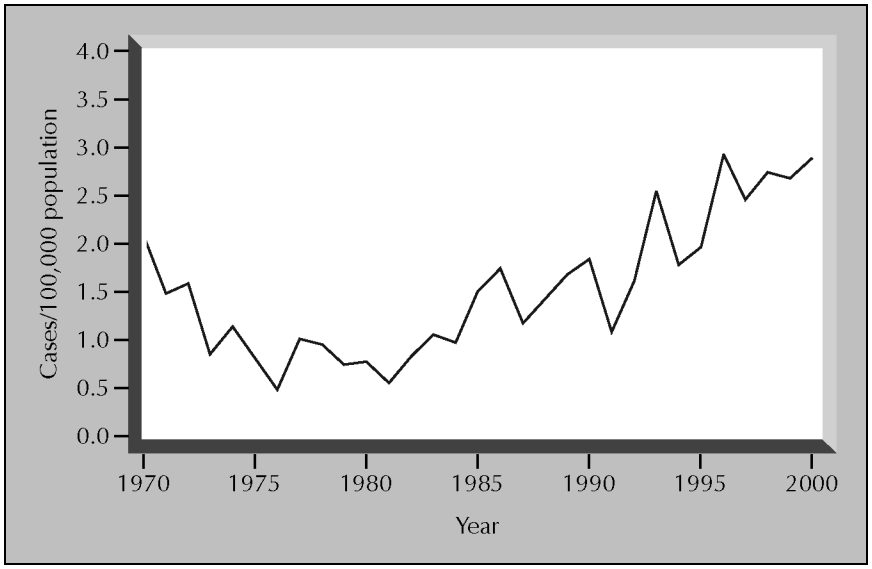


**Figure 1.** Reported incidence of pertussis in the United States, 1970 through 2000. Cases (per 100,000 population) have steadily increased in the past three decades. (*Adapted from* www.cdc.gov; accessed December, 2002.)

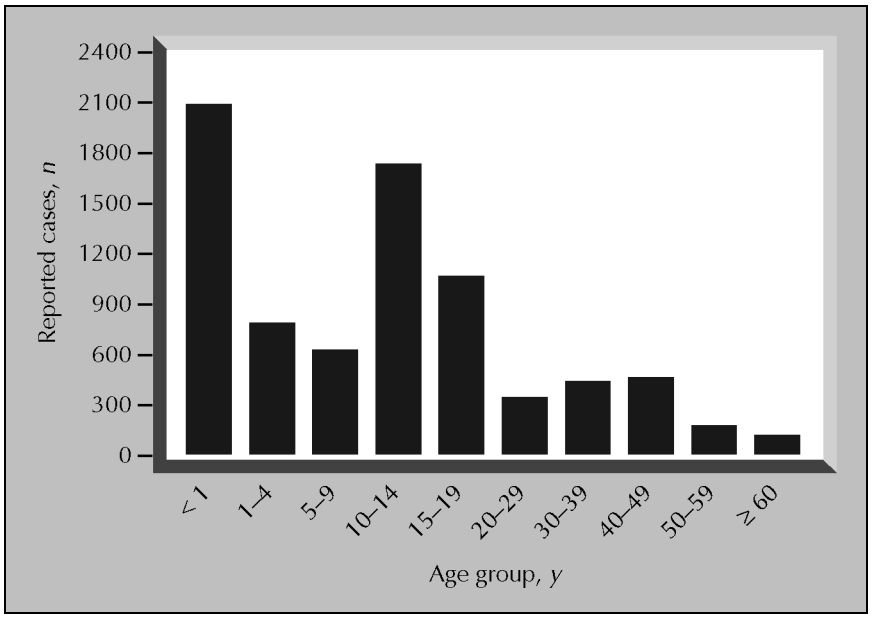


**Figure 2.** Reported cases of pertussis by age group in the United States, 2000. (*Adapted from* www.cdc.gov; accessed December, 2002.)

## Diagnosis

Infection with pertussis results in a wide spectrum of clinical manifestations, depending on the age and immune status of the host. Asymptomatic to mild coughing illness is common in adolescents and adults. Characteristic paroxysms of cough with an end inspiratory whoop occur in children, and a nonspecific coughing illness with apnea and cyanosis but no whooping is frequently observed in infants. A high index of clinical suspicion and knowledge of the local epidemiology are necessary for a prompt and accurate diagnosis.

Laboratory tests should be used in conjunction with clinical symptoms for confirmation of the diagnosis of pertussis. However, negative tests cannot completely rule out the diagnosis. An increased peripheral white blood cell count with lymphocytosis is characteristic but not specific. The direct fluorescent antibody assay lacks both sensitivity and specificity and is not a confirmatory test, but may provide support when culture is negative and other laboratory assays are unavailable. Detection of antibodies to specific pertussis antigens by enzyme-liked immunosorbent assay can be sensitive and specific, but it is not practical in the clinical setting. Diagnostic criteria typically include significant rises in titer to one or more pertussis antigens and/or a single high titer of antibody. In immunized individuals, a rise in immunoglobulin (Ig)A antibodies against whole cell *Bordetella pertussis*, pertussis toxin, filamentous hemagglutinin, or pertactin, has been proposed as and indicator of recent infection. Although specific, IgA measurement also lacks sensitivity (reported range, 24%–64%) [16].

The preferred test for laboratory confirmation of pertussis is now the detection of *B. pertussis* DNA by

**Table 1. Advantages and disadvantages of different methods of diagnosis of pertussis**

| Diagnostic method | Advantage | Disadvantage |
|---|---|---|
| Culture | Provides definitive diagnosis<br>High specificity > 95%<br>Allows antibiotic susceptibility testing and DNA fingerprinting | Variable sensitivity depending on age of patient, method of sample collection, timing of illness, use of adequate media, and transport<br>Takes several days<br>Media not generally available |
| DFA | Rapid results | Low sensitivity and specificity<br>Cross reactivity with normal nasopharyngeal flora |
| Serology<br>IgG/IgA ELISA | Specific | Low sensitivity due to duration and insidious onset of disease<br>Requires serum samples<br>Delayed diagnosis, not clinically helpful |
| PCR<br>Conventional<br>TaqMan*<br>LightCycler† | At least as sensitive as culture<br>High specificity > 95%<br>Rapid results (usually < 48 h) | Not standardized/validated methods<br>False-positives due to contamination<br>Quality assurance and trained personnel are necessary |

*Applied Biosystems, Mississauga, Ontario, Canada
†Roche Diagnostics, Hilden, Germany
DFA—direct fluorescent antibody; ELISA—enzyme-linked immunosorbent assay; Ig—immunoglobulin; PCR—polymerase chain reaction.

polymerase chain reaction (PCR) assays (Table 1). Bacteriologic culture provides a definitive diagnosis but it is usually not feasible as media formulations are not commercially available and *B. pertussis* is difficult to isolate from suboptimal specimens. The CDC recommends that culture be obtained whenever PCR is performed [17]. The reported sensitivity of culture varies from 6% to 95%, and that of PCR from 21% to 98%; both tests have a high specificity, exceeding 95%. Calcium alginate swabs can inhibit PCR results; therefore, dacron and rayon swabs are preferred for the collection of nasopharyngeal samples for both PCR and culture [18]. The sensitivity of these tests is higher if the sample is collected early during the clinical course of pertussis. Three to 10 days may be needed to isolate and identify *B. pertussis* by culture, while PCR results can be obtained within a few hours. However, only culture isolates allow for antibiotic susceptibility testing and nucleic acid fingerprinting for epidemiologic studies and outbreak investigations [17]. Caution should be applied when using PCR to detect outbreaks of pertussis. Results must be interpreted in the context of clinical illness because there is a potential for overdiagnosing pertussis due to the occurrence of false positives associated with DNA contamination in the laboratory [19•]. PCR protocols and reagents require standardization and validation among laboratories, which must have adequately trained personnel and quality assurance programs in place.

Although direct detection of *B. pertussis* DNA in clinical samples using sensitive amplification methods is increasingly utilized for the diagnosis of pertussis, there is variability in the sensitivity of the different PCR assays. Direct comparisons of studies using PCR diagnosis are difficult, given that different DNA purification techniques, PCR primers, reaction conditions, and product detection methods are used. Conventional PCR protocols target different regions of the *B. pertussis* genome, including the insertion sequence IS481, the pertussis toxin promoter region, the adenylate cyclase gene, and the porin gene. Multiplex PCR assays include targets for both *B. pertussis* (IS481) and *Bordetella parapertussis* (specific insertion region IS1001, adenylate cyclase gene, nested pertussis toxin promoter region), but do not distinguish between the two. *Bordetella holmesii* shares the IS481 region of *B. pertussis* and false-positive results may occur in patients colonized with this bacterium, although its role as a cause of upper respiratory illness has not been defined [20]. More recently, PCR assays that allow faster, real-time detection of the amplification product have been developed. TaqMan (Applied Biosystems, Mississauga, Ontario, Canada) and LightCycler (Roche Diagnostics, Hilden, Germany) technologies for PCR assays have been reported to increase the diagnostic sensitivity of culture by at least twofold [20,21]. In one study, TaqMan PCR was reported to have a sensitivity of 100%, a specificity of 97.4%, a positive predictive value of 87.6%, and a negative predictive value of 100% when compared with culture (11.6%, 100%, 100%, and 85.7%, respectively) in clinical samples obtained with a single nasopharyngeal swab of patients with symptoms of pertussis or who had been in contact with a case of pertussis [22]. Duplex LightCycler PCR assays (*ie*, allowing the detection of both *B. pertussis* and *B. parapertussis*) may result in lower sensitivity than simplex assays (for *B. pertussis* only), and are not currently recommended for clinical specimens [21].

## Prevention

Virulence factors associated with *B. pertussis* include adhesion molecules (filamentous hemagglutinin [FHA], pertactin [PRN], BrkA, and fimbriae) and toxins (pertussis toxin [PT], tracheal cytotoxin, adenylate cyclase toxin, dermonecrotic toxin). PT, FHA, PRN, and fimbrial agglutinogens (FIM) have been shown to elicit immune responses

in humans, although optimal protective levels are not known. Acellular pertussis vaccines containing only PT have been shown to be effective in prevention of pertussis, suggesting that immunity to PT is sufficient [23]. The presence of serum antibodies to PT is the most accepted method to assess the adequacy of the immune response to vaccination. Some experts believe that multicomponent acellular vaccines containing other antigens of *B. pertussis* (FHA, FIM, PRN) in addition to PT may be more effective. These purified antigens are included in pertussis vaccines in different amounts and combinations. However, available data are insufficient to directly compare the effectiveness of currently available licensed acellular pertussis vaccines.

In the United States, acellular pertussis vaccines have replaced whole cell pertussis vaccines for routine childhood immunization. There are five diphtheria-tetanus-acellular pertussis combination vaccines licensed in the United States, but only three are commercially available. Tripedia (Aventis Pasteur, Swiftwater, PA) and Infanrix (GlaxoSmith-Kline, Philadelphia, PA) are now licensed for all five doses in the primary immunization series. A fifth acellular pertussis vaccine (DAPTACEL, Aventis Pasteur, Toronto) was approved in the United States in 2002 for administration in the first four doses [24]. Tripedia contains PT and FHA; Infanrix contains PT, FHA, and PRN; and DAPTACEL is a five-component vaccine with PT, FHA, fimbria agglutinogens 2 and 3, and PRN. Because of the reduced frequency of adverse reactions and demonstrated efficacy, the Advisory Committee on Immunization Practices recommends a licensed DTaP for all five doses of the routine childhood immunization series [2]. There are no data to demonstrate superiority of one vaccine preparation versus the others, but whenever possible, the same vaccine formulation should be used for the entire series. The interchangeability of two DTaP vaccines, Tripedia and Infanrix, for the primary immunization series was recently evaluated by Greenberg *et al*. [25•]. Immune responses were assessed in infants receiving Tripedia at 2, 4, and 6 months of age (control group) versus infants receiving Tripedia at 2 and 4 months of age followed by Infanrix at 6 months, or Tripedia at 2 months followed by Infanrix at 4 and 6 months of age. Responses to PT, FHA, and PRN by enzyme immunoassay were 97% to 100% after the three-dose series in all three groups. Postlicensure studies demonstrate the safety of acellular pertussis vaccines, although a higher rate of injection site reactions requiring medical evaluation has been associated with the fourth and fifth doses of DTaP, particularly if the primary series consisted of DTP [26,27•].

Modified formulations of acellular pertussis vaccines for immunization of susceptible adolescents and young adults are undergoing active evaluation. Initial clinical trials carried out in the US in the 1990s have shown the vaccines to be safe, immunogenic, and well tolerated, with relatively limited local side effects, such as mild pain, redness, and induration [28]. The efficacy of acellular pertussis vaccines in the prevention of laboratory confirmed pertussis coughing illness in adults was 78% in a multicenter trial [29]. Adult formulations of a combined acellular pertussis vaccines have been licensed in Canada (ADACEL, Aventis Pasteur) [30] and in Europe (Boostrix, GlaxoSmithKline Biologicals). These vaccines contain a reduced concentration of diphtheria and pertussis components (dTap) when compared with the vaccine licensed for use in infants (DTaP) [31]. The pertussis antigens included in ADACEL are PT (2.5 µg), FHA (5 µg), fimbriae agglutinogens 2 and 3 (5 µg), and PRN (3 µg), while Boostrix contains PT (8 µg), FHA (8 µg), and PRN (2.5 µg). ADACEL is currently recommended for children over 7 years of age who have not had a primary pertussis immunization or for whom the immunization status is unknown. Adolescent booster doses with Boostrix are now recommended in Europe. Monocomponent acellular pertussis vaccines are also undergoing research for use in adolescents and adults.

Other combination vaccines are licensed in Europe and Canada. The current trend is for manufacturers to develop and research acellular pertussis vaccines in combination with other components such as *Haemophilus influenzae* type b vaccine (Hib), inactivated polio vaccine, and hepatitis B vaccine [32–38]. The main considerations when using these vaccines are the potential for increased local and systemic side effects with a greater number of antigens delivered, and interference of some of these antigens with the immune responses to others (*eg,* Hib). Different methods of delivery are also being studied including mixing of vaccines in a single injection or concomitant administration of vaccines in different sites.

Neonates and unimmunized infants are more susceptible to pertussis, suggesting that maternal antibodies transmitted to the infant transplacentally are insufficient to protect against disease. This may be a reflection of the waning immunity of women of childbearing age. Boosting the levels of antibodies that can be transported through the placenta by immunizing women with pertussis vaccine during pregnancy is an alternative approach for the prevention of pertussis in young infants that deserves further research. All the deaths associated with pertussis infection reported in the United States in the year 2000 occurred in infants less than 4 months of age. The immaturity of the neonatal immune system and the lack of safe and more immunogenic vaccines for administration in the neonatal period make maternal immunization an attractive intervention.

## Treatment

Recommended treatment regimens for pertussis are described in Table 2. Erythromycin for 14 days is the treatment of choice for all age groups. If administered during the early stages of pertussis (catarrhal or early paroxysmal), erythromycin may modify the clinical disease. When initiated later, treatment may not lessen the duration or severity of illness, but it may reduce the risk of spread to others. Erythromycin for 14 days is also the regimen of choice for

**Table 2. Treatment and prophylaxis regimens for pertussis**

| Antibiotic | Age group | Dosage | Duration |
|---|---|---|---|
| Erythromycin | Children | 40–50 mg/kg/d div q 6 h PO* or IV | 14 days |
| | Adults | 250–500 mg div q 6 h PO or IV | 14 days |
| Trimethoprim-sulfamethoxazole | Children | 8/40 mg/kg/d div q 12 h PO | 14 days |
| | Adults | 160 mg/800 mg q 12 h PO | 14 days |
| Clarithromycin | Children | 15–20 mg /kg/d div q 12 h PO | 10–14 days |
| | Adults | 250–500 mg q 12 h PO | 10–14 days |
| Azithromycin | Children | 10–12 mg/kg/d q d PO | 5–7 days |
| | Adults | 500 mg on day 1 and 250 mg/d on subsequent days | 5–7 days |

*The estolate preparation is often recommended because it is more acid stable and achieves higher serum and tissue concentrations.
div—divided; IV—intravenous; PO—oral; q—every.

prophylaxis in contacts of individuals with pertussis infection. Erythromycin commonly is associated with gastrointestinal side effects and an increased incidence of pyloric stenosis in infants. Alternative drugs for patients who cannot tolerate erythromycin include trimethoprim-sulfamethoxazole and the newer macrolides azithromycin and clarithromycin. These two antibiotics have demonstrated in vitro activity against *B. pertussis*, and when administered at standard doses for a shorter duration (5–7 days) they have been found to be as effective as a 14-day course of erythromycin for the treatment of pertussis in children as young as 1 month of age [39,40].

Erythromycin resistance in *B. pertussis* was first described in 1994 in the United States [41]. Treatment with trimethoprim-sulfamethoxazole resulted in microbiologic and clinical cure in this 2-month-old infant. Only three other resistant isolates have been identified since [42,43]. In the United States, the estimated rate of erythromycin resistance is less than 1% [43]. National surveillance to monitor for resistant *B. pertussis* isolates and their potential association with changes in pertussis epidemiology is ongoing, but routine antimicrobial susceptibility testing of *B. pertussis* isolates is not currently recommended. Although a standardized screening method is not available, disk diffusion and E-test assays on charcoal-horse blood agar or Regan-Lowe agar with or without cephalexin plates are considered adequate screening tests for antibiotic resistance [43,44]. *B. pertussis* strains with a heterogenous phenotype have been recently described [43]. These isolates show growth of resistant colonies appearing inside an initial zone of inhibition after an extended incubation period of 5 to 7 days. Studies are underway to attempt to elucidate the mechanism of erythromycin resistance in *B. pertussis*.

Finally, in October 2002, a case of a clinical recurrence of culture-confirmed pertussis was reported in a 2-month-old infant treated with azithromycin (10 mg/kg/d for 10 days). It is unclear whether the recurrence was associated with antibiotic failure because the child also received steroids during the initial episode, and definitive tests were not done to confirm that the recovered organisms were the same strain [45].

## Conclusions

Pertussis has re-emerged worldwide as an important cause of morbidity and mortality in pediatrics despite the availability of efficacious vaccines and high rates of immunization in developed countries. More adolescents and young adults are suffering from pertussis illness as the immunity provided by childhood immunization wanes. A booster pertussis vaccination for adolescents is the most readily available solution to decrease the incidence of pertussis in this age group and may have the added advantage of interrupting transmission to unprotected infants who also have the greatest risk of mortality from the disease. Although laboratory confirmation of pertussis remains a challenge, rapid nucleic acid amplification assays are being used with increasing frequency in clinical laboratories, providing the opportunity to diagnose pertussis early in its course and initiate antibiotic therapy to prevent further spread to susceptible individuals.

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- •• Of major importance


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Despite the effectiveness of vaccination, pertussis continues to occur in the United States among all age groups, and the rates of disease continue to increase in a trend that began in the early 1980s. This has prompted the study of the burden of pertussis among children, adolescents, and adults, and the continuation of such trend is likely to impact on the use of acellular pertussis vaccines among persons 7 years of age and older.

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