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Rapid Diagnosis of Pertussis in Young Infants: Comparison of Culture, PCR, and Infant's and Mother's Serology

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The contribution of maternal pertussis serology comparing prepartum serum to serum collected during the infant's disease to the diagnosis of pertussis in infants was evaluated for 28 pairs of young infants with pertussis syndrome and their mothers and was compared to those of culture and PCR. Infants had a nasopharyngeal aspiration tested by PCR, and acute and convalescent sera were collected during their disease. Mothers had a first acute serum collected concomitantly with the infant's acute serum, and both acute sera were compared to a prepartum serum. Sera were analyzed by immunoblotting for the detection of anti-pertussis toxin (PT) antibodies. Serological evidence of pertussis in infants was assessed as either an increase in anti-PT antibody levels between the mother's prepartum and acute sera or the presence of antibodies in the infant's acute serum and their absence in both the mother's acute and prepartum sera. Culture and PCR sensitivity were 43 and 89%, respectively. Most infants (18 of 24) had no pertussis antibody detectable in their acute sera, confirming a delayed immune response at this age. A comparison of infant's and mother's serology, using prepartum serum, rapidly confirmed the diagnosis in 57% of the cases. Although less sensitive than PCR, this serological method should be used for a rapid diagnosis of pertussis in young infants when culture and PCR are either not available or negative.

Prevention of pertussis, a highly contagious respiratory disease, is accomplished by vaccination. In France, a single whole-cell pertussis vaccine (Pasteur Mérieux Sérums et Vaccins) has been used since 1957 and has been combined with the diphtheria, tetanus, and polio vaccines since 1966. The French vaccination schedule consists of three injections, at 2, 3, and 4 months of age (since 1991), and a single booster at 18 months. A high level of vaccination coverage has been maintained for more than 30 years, and as a result, the incidence of pertussis has decreased dramatically for several decades; however, a resurgence has recently been suspected (1). This phenomenon is similar to that observed since 1976 in the United States (3) and is attributed in France to the progressive diminution of vaccine-induced protection because of the absence of a late booster, since no decrease in vaccination coverage or in vaccine efficacy has been observed (2). As a consequence, most of the cases are now seen in susceptible populations, such as very young unimmunized infants and vaccinated adolescents and adults (1, 2).

Pertussis diagnosis remains difficult in many pediatric settings or in private practice because clinical presentation may be atypical (8), especially in immunized patients and young infants, and laboratory confirmation is still problematic (14). However, rapid confirmation of pertussis is useful, especially for parents of very young infants (less than 6 months of age) who are hospitalized for severe clinical pertussis and for clinicians, who will decide to treat the household prophylactically.

Culture sensitivity does not exceed 60% at best and decreases rapidly with time after the onset of paroxysms or the start of antibiotic treatment (7, 17). PCR sensitivity is superior to that of culture but is also reduced in patients tested late in their disease (16). Furthermore, no standardized PCR method has been universally recommended to date (12).

When culture or PCR is not available, or is negative, specific pertussis serology can be used as an alternative method, by either enzyme-linked immunosorbent assay or immunoblotting (6). This method is usually retrospective, since it requires the comparison of paired sera collected at 4-week intervals, and thus is not compatible with a rapid diagnosis. However, a serological method for rapid diagnosis has been described in which antibody levels of single convalescent-phase sera were compared to those of pooled sera from a control population (9, 15). This methodology does not seem to be appropriate for young infants for several reasons: serological immune response is frequently delayed at this age compared to that in older patients, some young infants with pertussis may be partially vaccinated, and maternal antibodies may persist until 6 months of age.

Another way to confirm pertussis in coughing individuals is by demonstration of contact with a person with a culture-confirmed case (epidemiological linkage) (18). To date, this method is restricted to vaccine studies and is not accepted when PCR or serology are used to confirm pertussis in the contact cases. When young infants are infected by *Bordetella pertussis*, the index case is frequently identified among the household (13). A recent national epidemiological study performed in France of young infants suspected of pertussis showed that 34% of the index cases were identified among parents (1, 2). A previous study also conducted with young infants admitted for pertussis in a French hospital in Paris showed that 69% of the index cases were in mothers (5). During this study we observed that the comparison of an infant's acute serum with the mother's sera collected before delivery and early during the infant's disease can help to confirm a diagnosis of pertussis for young infants with clinical symptoms. In fact, we observed either the presence of anti-pertussis toxin (PT) antibodies in the infant's acute serum and

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their absence in the mother's prepartum and acute sera, implying that the infant's antibodies were not of maternal origin (in the absence of previous vaccination), or an increase of anti-PT antibodies in the acute maternal serum compared to the prepartum serum, regardless of the mother's symptoms, suggesting a recent contact by the mother with a person suffering from pertussis. We previously observed that such a seroconversion is seen earlier in mothers than in infants (5).

Prepartum sera are easily available in France, since all pregnant women are tested for rubella, syphilis, toxoplasmosis, and hepatitis B during pregnancy and laboratories are required by law to keep the serum sample for 1 year after collection.

The aim of the study was to compare the contributions of culture, PCR, and compared pertussis serology in infants and their mothers, using prepartum serum, to the rapid diagnosis of pertussis.

MATERIALS AND METHODS

Informed consent was obtained from parents or guardians. The study protocol followed the guidelines of the Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale according to present French legislation.

Patients. We selected 28 infants less than 6 months of age with confirmed pertussis and whose mothers' prepartum and acute sera were available. Confirmation of pertussis in the infants was provided by positive culture and/or PCR and/or serology in the infant or positive culture and/or PCR in the mother (epidemiological linkage) or both.

Symptoms were classified according to three clinical definitions: typical pertussis, defined as a prolonged cough, lasting more than 21 days, with paroxysms; atypical pertussis, defined as a cough lasting less than 21 days; and asymptomatic, defined as no respiratory symptoms. Information concerning immune status (from previous cases of pertussis or vaccination) was also collected.

A nasopharyngeal aspirate (NPA) was collected for culture and PCR during the first visit for all infants and some mothers. Two serum samples (acute [S1] and convalescent [S2]) were collected for most infants during the course of their disease, at 4-week intervals. From the mothers, a first serum (S1) was collected concomitantly with the collection of acute serum from the infants, and it was compared to a prepartum serum (S0). Some of the mothers also had a second serum (S2) collected 1 month after S1, regardless of their symptoms.

Culture and PCR assays. NPAs were collected in dry, sterile tubes during the first examination and were transported within 4 h at room temperature to the laboratory for culture. The samples were divided into three portions. One portion was plated on fresh Bordet-Gengou agar enriched medium (BGEM) and cultured at 36°C for 72 h. The second was inoculated in Stainer-Scholte medium for 20 h at 36°C before inoculation on BGEM (6). The remaining portion was stored at −80°C until PCR analysis. Identification of all isolates was biochemically verified by testing catalase, oxidase, urea, nitrate reduction, pigmentation on tyrosine agar, and growth on blood agar.

PCR amplification of the PT gene promoter region was done as previously described (4). Briefly, NPAs were digested with proteinase K and submitted to 40 cycles of amplification with the two primers derived from nucleotides 307 to 332 and 469 to 497 of the *pt* promoter (10). PCR products were transferred to a nylon membrane for Southern blot analysis. Hybridization was conducted with a fluorescein-labelled probe specific for the *B. pertussis pt* promoter, and final detection was performed with horseradish peroxidase-labelled anti-fluorescein antibodies (Amersham, Little Chalfont, United Kingdom) by using the Amersham enhanced chemiluminescence system.

Immunoblot assay. Serum detection of anti-PT antibodies was performed by Western blotting as previously described (6). Briefly, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed on ready-to-use 8 to 25% polyacrylamide gels with a Phast-system (Pharmacia, Uppsala, Sweden). The separated proteins were transferred on Hybond C super membranes (Amersham). After blocking, membranes were incubated overnight at 4°C with a 2×10^{-3} dilution of human serum. The detection of antibodies was performed with peroxidase-labelled anti-human immunoglobulins by using the Amersham enhanced chemiluminescence system. Results were graded, depending on the duration of exposure to X-ray films, as follows: 3, 6 s; 2, 1 min; 1, 10 min; and 0, no detection. Detection was considered positive when the result was 1, 2, or 3. Serological confirmation was determined by an increase in the detection of antibodies directed against PT between paired sera (seroconversion).

RESULTS

Twenty-eight infants and their mothers were included in the study.

Clinical data. (i) Infants. Clinical data for the infants are described in Table 1. The mean age of the subjects was 2.5 months (range, 0.6 to 6.3 months), and the sex ratio (male/female) was 0.86. The mean delay between the first symptoms and the collection of acute sera was 18.5 days (range, 6 to 48 days). All the infants had paroxysmal coughs at the time of their inclusion in the study which were considered, at follow-up, as typical of pertussis in 26 cases (93%) and atypical in 2 cases. Two of them had been vaccinated against pertussis, but they had received only one injection 10 and 30 days, respectively, before acute serum collection.

TABLE 1. Clinical data and bacteriological and serological results for infants

Family	Age (mo)	Vaccinations (no. of injections)	Cough[a]	Culture/PCR[b]	Anti-PT Ab (S1/S2)[c]
1	2.1	0	T	+/+	1/2
2	3.1	0	T	−/+	2/2
3	2.9	0	T	+/+	0/1
4	2.0	0	T	−/−	0/1
5	1.3	0	A	−/+	1/0
6	1.6	0	T	+/+	0/2
7	4.9	0	T	+/+	0/ND
8	2.8	0	T	−/+	0/ND
9	2.5	0	T	−/+	0/2
10	2.0	0	T	−/−	1/0
11	2.1	0	T	−/+	0/3
12	1.7	0	T	+/+	0/ND
13	1.8	0	T	+/+	1/ND
14	4.4	0	T	+/+	0/1
15	2.4	0	T	+/+	0/3
16	3.5	0	T	−/+	0/0
17	1.6	0	T	−/+	0/3
18	3.4	0	T	+/+	0/2
19	1.3	0	A	−/+	1/0
20	1.3	0	T	−/+	0/1
21	3.5	1	T	+/+	1/3
22	1.9	0	T	−/−	0/1
23	3.9	1	T	−/+	2/3
24	3.0	0	T	+/+	0/3
25	0.6	0	T	+/+	0/3
26	6.3	0	T	−/+	2/2
27	1.9	0	T	−/+	1/0
28	0.7	0	T	−/+	0/0

[a] T, typical of pertussis; A, atypical.
[b] +, positive; −, negative.
[c] Anti-PT Ab, anti-PT antibodies detected by Western blotting; S1, acute serum; S2, convalescent serum. Results were graded according to the length of exposure to X-ray film as follows: 3, 6 s; 2, 1 min; 1, 10 min; and 0, no detection. ND, not done.

(ii) Mothers. Clinical data for the mothers are described in Table 2. The mean age was 29.4 years (range, 19.2 to 42 years). Nine of them (32%) had typical pertussis, 9 (32%) had atypical pertussis, and 10 (36%) were asymptomatic. Two mothers remembered having a past infection, and seven were immunized (four to five injections). Of these nine mothers, six were asymptomatic and three had an atypical course of infection. Among the 19 mothers who had no memory of pertussis or pertussis immunization, 9 had typical pertussis, 6 had atypical pertussis, and 4 were asymptomatic.

Culture and PCR results. For the infants, the respective rates of positive culture and positive PCR were 43 (12 of 28) and 89% (25 of 28). NPAs were collected from 18 mothers and cultured on BGEM, but only 15 PCRs were performed. Cultures were positive in 4 (22%) and PCRs were positive in 8 (53%) of 15 cases, of which 1 was asymptomatic. No relation was found between the age of the mother and culture or PCR positivity rates.

TABLE 2. Clinical data and bacteriological and serological results for mothers

Family	Age (yr)	Time delay (mo) between S0 and S1	Vaccinations (no. of injections) or history of pertussis (age)	Cough[a]	Culture/ PCR[b]	Anti-PT Ab S0/S1/S2[c]
1	30.95	4.2	5	N	−/−	0/2
2	19.23	5.9	U	N	−/−	0/0/0
3	34.95	2.8	4	A	+/+	0/0/2
4	34.39	7.9	U	T	ND/ND	0/3
5	23.89	2.2	U	N	ND/ND	1/1
6	35.47	5.7	U	T	−/−	0/1
7	28.29	5.8	U	A	−/ND	0/0
8	29.81	2.9	4 yr	N	ND/ND	0/0
9	41.99	8.9	1 yr	N	−/ND	0/0
10	35.23	7.2	U	T	−/+	2/2
11	25.07	7.7	5	N	ND/ND	0/0/1
12	U[d]	2.0	U	N	ND/ND	0/1
13	23.55	1.6	U	T	−/+	0/0/1
14	29.67	4.1	U	T	+/+	1/3/3
15	22.09	9.7	4	A	−/+	0/2/3
16	28.65	5.6	U	N	−/ND	0/2
17	36.21	10.4	U	T	+/+	0/0/0
18	21.78	9.4	U	T	ND/ND	0/2/3
19	27.48	7.9	U	A	ND/ND	1/1/1
20	26.70	11.7	U	A	ND/ND	0/2/2
21	21.96	5.5	4	A	−/−	0/1/3
22	20.42	7.9	U	A	ND/ND	0/0/1
23	35.78	7.1	U	A	−/−	0/1
24	29.98	6.5	5	N	−/+	0/3/3
25	40.22	1.3	U	T	ND/ND	0/3/3
26	34.67	6.3	U	T	−/−	0/2
27	26.01	9.5	4	N	−/−	2/2/2
28	29.55	7.6	U	A	+/+	0/1/2

[a] T, typical of pertussis; A, atypical; N, no cough.
[b] +, positive; −, negative; ND, not done.
[c] Anti-PT Ab, anti-PT antibodies detected by Western blotting; S0, prepartum serum; S1, acute serum; S2, convalescent serum. When only two numbers are given, they represent S0 and S1. Results were graded according to the length of exposure to X-ray film as follows: 3, 6 s; 2, 1 min; 1, 10 min; and 0, no detection.
[d] U, unknown.

Serological results. Only 24 infants had paired sera collected at 1-month intervals (Table 1). The mean delay between the onset of coughing and an infant's acute serum collection was 18.5 days (range, 6 to 48 days; median, 14.6 days). Most infants (18 of 24) had no pertussis antibody detectable in their acute sera; however, a seroconversion between acute and convalescent sera was observed in 16 infants (67%). Such serological confirmation was more frequent after the age of 2 months (75 versus 50%). For the other eight infants the comparison of the two sera showed either a decrease (four cases) or no change (two cases) in anti-PT antibody level or no detection of anti-PT antibody (two cases).

All the mothers had serum (S1) collected early during the disease of their child, regardless of their own symptoms (Table 2). This serum was tested concomitantly with a prepartum serum (S0) collected during pregnancy. The mean delay between collection of S0 and S1 was 188 days (range, 40 to 352 days). Seroconversion was observed between S0 and S1 in 15 cases (53%); among these mothers, 4 were asymptomatic. Anti-PT antibodies were detected, but no change between S0 and S1 was observed, in four mothers; two were asymptomatic. No anti-PT antibody was detected in either S0 or S1 in the last nine mothers, of whom five were coughing at S1 collection. No difference in serological results was observed based upon immune status.

A comparison of late convalescent serum (S2) with acute serum (S1) was performed for 15 mothers, with the following results: late seroconversion, seven cases (of which four had no anti-PT antibody detected in S1); no change, eight cases (of which two had no anti-PT antibody detected in S1 and S2).

Comparison of maternal and infant serology. Ten infants had anti-PT antibodies already detectable in their acute sera. In four of them, the convalescent sera showed no anti-PT antibody, thus suggesting a maternal origin, as was confirmed by the analysis of the mothers' prepartum sera, which showed the presence of anti-PT antibodies. Among the six other infants, evidence of recent infection, as suggested by the absence of anti-PT antibody or by the presence of lower antibody levels in both the mothers' S0 and S1 sera, was found in three cases.

DISCUSSION

When culture and PCR are available, direct identification of *B. pertussis* is the most rapid and sensitive method for diagnosis. In our study, PCR sensitivity was twice that of culture, for both infants and adults. However, PCR was less sensitive for adults than for young infants, confirming our previous results (4) and those of a recent study showing that PCR sensitivity decreases with the age of the patient (16), probably due to an accelerated clearance of the bacteria.

Pertussis diagnosis was rapidly confirmed for 16 of 28 infants (57%), using the strategy of comparing maternal serology to infant's serology. In 15 cases, an increase in anti-PT antibodies was observed between prepartum and acute sera, and for 3 cases, anti-PT antibodies were detected in the infants' acute sera but not in the maternal prepartum or acute sera. Furthermore, for one patient (family case 4), culture and PCR were negative, although the infant's disease was typical and diagnosis was confirmed by this serological method. However, compared to PCR, the sensitivity of this method was only 64% (16 of 25), and its applicability depends essentially on the availability of prepartum sera (which may not be possible in other countries).

In our study, most infants had no anti-PT antibody detectable in their acute sera despite a relatively long duration of coughing, confirming that the immune response may be delayed in infants compared to that in children and adults. Moreover, seroconversion rates were positively related to the ages of the infants. In very young infants, less than 2 months of age, the seroconversion rate between paired sera collected at 1-month intervals was only 50%, confirming that neither conventional infant's serology nor rapid diagnosis by using acute serum is appropriate at this age. However, we restricted our serological analysis to anti-PT antibodies because of their absolute specificity in pertussis. The use of other pertussis antigens, such as filamentous hemagglutinin or adenylate cyclase-hemolysin, might have increased the sensitivity of our method and should be tested in this context.

In conclusion, PCR is the most rapid (2 days) and sensitive method, compared to culture (5 to 7 days) and serology (1 month), for pertussis diagnosis in young infants. However, the use of compared pertussis serology in young infants and mothers may be useful if culture and PCR are not available or are negative. This method appears to be sensitive, rapid (2 days), and contributive, even if the mother is asymptomatic, confirming previous studies which showed that immune adults may elicit antibody response to infection without symptoms after contact with a pertussis patient (11).

We propose a model (Fig. 1) which indicates the most appropriate technique for pertussis diagnosis in infants less than 6 months of age. It takes into account the requirements of

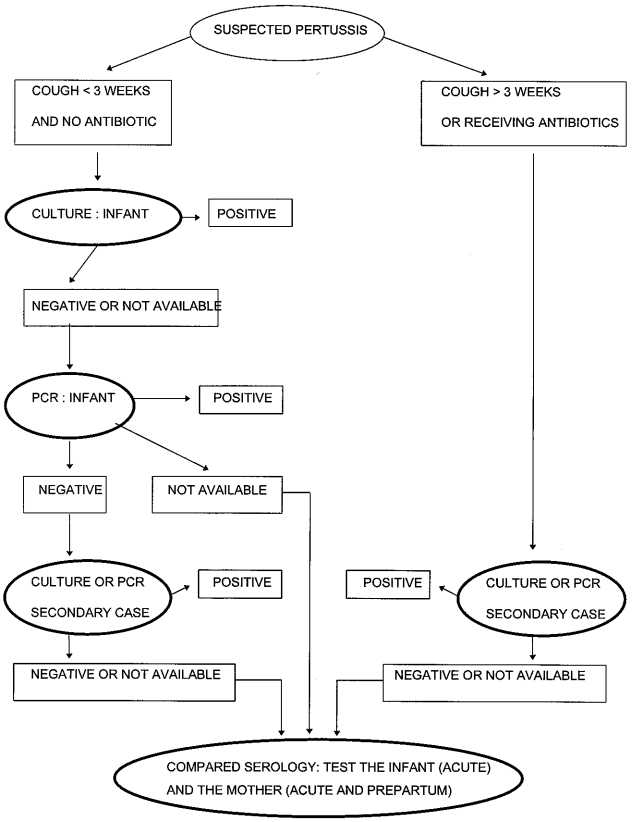

FIG. 1. Proposed model for the use of pertussis diagnostic techniques for infants less than 6 months of age.

rapidity, sensitivity, and minimum cost. When the infant has been coughing for less than 3 weeks and has not received any antibiotic effective against *B. pertussis* (such as erythromycin), culture should be performed first. Culture is less expensive than PCR, is absolutely specific, and should be continued in order to analyze the circulating strains, which are liable to vary in response to the generalized use of vaccine. PCR should be used first if culture is not available. When culture and PCR are negative or likely to be negative (e.g., the subject has been coughing for less than 3 weeks or has been treated with erythromycin) or are not available for an infant, they should be performed (if possible) on untreated secondary cases, since these two techniques are more likely to be positive with a reduced delay after the onset of infection. Finally, when culture and PCR are either negative or not available, compared serology of the infant and mother should be performed, since it is a rapid and sensitive way to confirm the diagnosis of pertussis.

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