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Efficacy of Enzyme-Linked Immunosorbent Assay for Rapid Diagnosis of *Bordetella pertussis* Infection

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We examined the diagnostic efficacy of an enzyme-linked immunosorbent assay (ELISA) for class-specific antibodies to *Bordetella pertussis* in acute-phase sera collected from 1,240 patients with suspected pertussis. A total of 833 serum specimens (67%) yielded positive results. The proportion of positive results increased to 77% if a second (convalescent-phase) serum was also tested. By comparison, a bacterial agglutination test for *B. pertussis* antibodies was positive in only 21% of acute-phase specimens and 50% of paired specimens. The high proportion of acute-phase sera which were ELISA positive indicates that a measurable serologic response has usually occurred by the time the diagnosis is suspected. Thus, the ELISA is potentially the most rapid means of laboratory confirmation of *B. pertussis* infection.

Whooping cough (pertussis) is a respiratory tract disease caused by *Bordetella pertussis*. In developing countries, high mortality rates are associated with whooping cough, particularly in infants and young children, whereas in developed countries, such as Australia, morbidity rates remain a cause of concern. Indeed, there is evidence that in Adelaide, as well as in several other major Australian cities, the incidence of the disease is increasing (the number of admissions to the Adelaide Children's Hospital for pertussis has trebled since 1982). This is despite the fact that approximately 95% of Adelaide children receive combined diphtheria-tetanus-pertussis vaccine during their first year of life (5).

During the early (catarrhal) stage of the illness, large numbers of *B. pertussis* organisms may be present in the nasopharynx. However, by the time the classical symptoms of whooping cough become apparent, the numbers are decreasing, and examination of nasopharyngeal smears by immunofluorescence or culture for *B. pertussis* frequently yields negative results. Failure to diagnose pertussis early in the course of illness has important consequences for infection control, because there is a high probability that undiagnosed individuals could transmit the infection to siblings and other close contacts, such as children in schools or day-care centers, or to nearby patients in the case of hospitalized individuals.

Serologic techniques therefore play an important role in the diagnosis of pertussis. Since symptoms sufficiently serious to warrant attendance at or admission to a hospital may develop only after the infection has been established for several weeks, a measurable serologic response might already have occurred at the time of presentation. Thus, sensitive serologic tests have the potential to provide the most rapid diagnosis of the disease. We investigated the use of an enzyme-linked immunosorbent assay (ELISA) which detects immunoglobulin A (IgA), IgG, and IgM to *B. pertussis* (in comparison with a bacterial agglutination [BA] test) for the rapid diagnosis of whooping cough. The ELISA was clearly superior to the BA test and resulted in positive diagnoses with 67% of all acute-phase serum specimens tested.

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MATERIALS AND METHODS

Bacterial strains. *B. pertussis* NCTC 10908 and NCTC 10911 (phase I; serotypes 1,2,3,4 and 1,2,5,6, respectively) were grown on charcoal agar (Oxoid Ltd., Basingstoke, England) supplemented with 20% horse blood. The organisms were harvested after incubation for 2 days at 35°C in a humid atmosphere.

Serum specimens. Single acute-phase serum specimens were obtained on initial presentation from 1,240 patients (age range, 1 month to 73 years; median age, 6 years) who had clinical signs of pertussis (e.g., chronic or paroxysmal cough with or without associated vomiting, etc.). A second serum specimen was also obtained from 129 of these patients approximately 2 weeks after collection of the first specimen to permit testing of paired specimens.

Serum was also collected from 100 patients who had been admitted to the Adelaide Children's Hospital for reasons unrelated to a respiratory complaint (generally elective or nonelective surgery) (age range, 1 month to 21 years; median age, 9 years). These sera were tested to determine the level of pertussis antibody in normal (i.e., uninfected) individuals. All sera were stored at −20°C before being tested.

ELISA for IgA, IgG, and IgM to *B. pertussis*. Freshly harvested bacteria were suspended in 0.1 M sodium carbonate (pH 9.6) such that the A_{600} was approximately 1.0. The suspension was incubated at room temperature overnight with 0.1% (vol/vol) formaldehyde and then sonicated for 60 s at 100 W. Samples of the sonic extract (100 μl) were then added to alternate columns of wells of flat-bottomed polystyrene microdilution plates (Dynatech Laboratories, Inc., Plochingen, Federal Republic of Germany). An equal volume of 0.1 M sodium carbonate (pH 9.6) was added to the wells in the remaining columns. After incubation at 4°C overnight, the plates were washed three times with phosphate-buffered saline containing 0.05% (vol/vol) Tween 20 (PBS-Tween) and then dried. The coated plates could be stored in sealed plastic bags at 4°C for up to 4 weeks before use.

Immediately before use, the plates were blocked by the addition of 150 μl of PBS-Tween containing 1% (wt/vol) bovine serum albumin and 1% (vol/vol) normal goat serum (PBS-Tween-BSA-GS) to each well, followed by incubation

TABLE 1. ELISA and BA analysis of single (acute-phase) sera[a]

BA result	No. of ELISA results						
	Negative	Positive	Total	Positive for:			
				IgG only	IgM only	IgA only	2 or more immunoglobulin classes
Negative	394	591	985	106	51	43	391
Positive	13	242	255	26	3	5	208
Total	407	833	1,240	132	54	48	599

[a] Sera were assayed as described in Materials and Methods.

at 37°C for 2 h and washing in PBS-Tween. Test sera were routinely diluted 1:400, 1:200, and 1:100 in PBS-Tween-BSA-GS for determination of anti-*B. pertussis* IgG, IgM, and IgA, respectively. Duplicate 100-μl samples of the diluted test sera were added to both antigen-coated and uncoated (control) wells for each immunoglobulin class to be assayed. Reference standards and negative control sera were also included in each plate. The plates were incubated for 2 h at 37°C in a humid atmosphere and then washed three times in PBS-Tween. Horseradish peroxidase-conjugated goat anti-human IgG, IgM, and IgA (γ-, μ-, and α-chain specific, respectively) were obtained from KPL Laboratories Gaithersburg, Md., and were routinely diluted 1:2,000, 1:1,000, and 1:500, respectively, in PBS-Tween-BSA-GS before use. Diluted conjugate (100 μl) was then added to the appropriate wells, and the plates were incubated at 37°C for 2 h and washed three times in PBS-Tween. Peroxidase substrate solution (100 μl; Bio-Rad Laboratories, Richmond, Calif.) was then added to all wells, and after incubation for 30 min at 30°C the A_{415} of each well was measured with a Uniskan ELISA reader (Labsystems, Helsinki, Finland).

A test result for a particular serum specimen was considered acceptable only if the A_{415} of respective non-antigen-coated wells was less than 0.35 and the A_{415}s of the reference standard sera were ≥1.5 (the A_{415}s of negative control sera were always <0.05). The reference standard sera for IgG, IgM, and IgA assays were pooled human sera which had been tested previously and found to be strongly positive for the respective anti-*B. pertussis* immunoglobulin class but negative for the other two classes. For each test serum, the A_{415} was expressed as a percentage of the A_{415} for the respective reference standard serum. An ELISA result was defined as positive if this value exceeded the mean value for the normal population by more than two standard deviations. By this criterion, the critical values for positive IgG, IgM, and IgA results were ≥51, ≥67, and ≥31%, respectively (the mean values for the normal population were 20, 35, and 12%, respectively). The mean ± standard deviation values for ELISA-positive sera from patients were 88 ± 9, 105 ± 23, and 68 ± 27% for IgG, IgM, and IgA, respectively.

BA test. The BA test was performed by adding serial twofold dilutions of test serum (prepared in saline) to an equal volume of fresh *B. pertussis* suspension (A_{600}, 0.5). Tubes were observed for agglutination after overnight incubation at 37°C. A test was defined as positive if the endpoint agglutination titer of an individual serum specimen was ≥256 or if there was a fourfold or greater change (increase or decrease) in titer between acute- and convalescent-phase sera.

RESULTS

Analysis of single serum specimens by ELISA and BA. The 1,240 acute-phase serum specimens were assayed for antibodies to *B. pertussis* by BA and ELISA and scored as positive or negative as described in Materials and Methods (Table 1). A total of 255 specimens yielded positive BA results, and 242 (95%) were also positive by ELISA. However, a further 591 (60%) of the 985 BA-negative specimens were positive by ELISA. Remarkably, 284 of these ELISA-positive specimens had undetectable BA titers (<4). Of the ELISA-positive, BA-negative specimens, 66% were positive by ELISA for at least two immunoglobulin classes, whereas of the ELISA-positive, BA-positive specimens, 86% were ELISA positive for at least two classes. Of the 833 ELISA-positive specimens, 83% had positive IgG values (either alone or in combination with positive IgM or IgA values), 73% had positive IgA values, and 46% were positive for *B. pertussis* IgM.

TABLE 2. ELISA and BA analysis of paired sera[a]

BA result	No. of ELISA results				
	Both sera negative	Serum 1 only positive	Serum 2 only positive	Both sera positive	Total
Negative	24	7	5	29	65
Significant change in titer	1	1	2	5	9
Positive titer in either serum	1	1	2	17	21
Significant change and positive titer	3	0	4	27	34
Total	29	9	13	78	

[a] Sera were analyzed as described in Materials and Methods.

Analysis of paired serum specimens. The above-described results highlight the superiority of the ELISA in the diagnosis of pertussis from a single serum specimen. However, serologic diagnosis by BA is also made on the basis of a fourfold change (increase or decrease) in titer between acute- and convalescent-phase sera; by this criterion, neither titer need necessarily exceed 128 for the result to be positive. Paired serum specimens were obtained from 129 patients and analyzed by ELISA and BA (Table 2). A total of 100 of the paired specimens were ELISA positive, and of these, 87 had a positive first specimen. However, the BA test detected only 64 positive paired specimens by all criteria, and diagnosis could have been made with the first serum alone in only 25 of these. Of the 65 paired specimens which yielded negative BA results, 41 (63%) were positive by ELISA (29 of these were ELISA positive for both serum specimens). Conversely, of the 29 paired specimens which were both negative by ELISA, only 5 yielded positive BA results.

BA and ELISA in culture-proven cases of pertussis. ELISA and BA results for sera from 58 patients with culture-proven pertussis are shown in Table 3. For the 45 patients over 4 months of age, 34 specimens were ELISA positive, but only 10 of these were BA positive. Serologic diagnosis was less

TABLE 3. ELISA and BA analysis of sera from culture-proven pertussis cases[a]

BA result	No. of ELISA results in patients			
	<4 mo of age (*n* = 13)		≥4 mo of age (*n* = 45)	
	Negative	Positive	Negative	Positive
Negative	9	2	11	24
Positive	1	1	0	10

[a] Acute-phase sera were analyzed as described in Materials and Methods.

effective in children under 4 months of age, however, since only 3 of the 13 specimens were ELISA positive and BA titers were positive for only two patients.

DISCUSSION

In this study, a high proportion (67%) of acute-phase serum specimens collected from patients with suspected pertussis were ELISA positive by our criteria. Such a high rate of positive specimens is consistent with the distinctive clinical picture of pertussis and may also have been contributed to by the fact that there was an epidemic of whooping cough in Adelaide at the time most of the sera were collected. The critical positive ELISA value (mean A_{415} of age-matched normal sera plus two standard deviations) was set such that statistically only 2.5% false-positives would be expected. Similar high positive rates have also been detected in other, albeit smaller, studies which used similar criteria (2, 6). In our study, the ELISA, as would be expected, had sensitivity vastly superior to that of the BA test, which detected only 21% of positive acute-phase specimens. When paired specimens were tested, the number of positive pertussis cases detected by BA increased to 50%, whereas testing a second convalescent-phase serum specimen by ELISA increased the proportion of specimens detected as positive by that method from 67 to 77%. One possible explanation for the high rate of false-negative BA results could be masking of agglutinogen 3 by agglutinogen 2. This would reduce the sensitivity of the BA test for sera from patients infected with, for example, *B. pertussis* serotype 1,3. Current data for the serotype distribution of *B. pertussis* in South Australia is not available. However, the ELISA, which uses extensively sonicated antigen rather than whole cells, would be less susceptible to this interference.

Some previous studies have reported the use of the ELISA for IgA to certain pertussis antigens (particularly the fimbrial hemagglutinin) for the diagnosis of whooping cough (2–4). Vaccination has been shown not to induce production of anti-pertussis IgA, whereas pertussis infection did (1). Thus, the IgA ELISA could distinguish people with genuine pertussis from recent vaccinees. In the present study, however, use of the IgA ELISA values alone would have missed 27% of the positive cases. We believe that background IgG and IgM levels in the general population as a result of prior immunization have been adequately accounted for by our test criteria and did not contribute to an unacceptably high level of false-positive specimens. In excess of 90% of both the control and study populations had been vaccinated. Thus, measurements of IgA, IgG, and IgM is clearly justified. To further investigate this, we are using the Western blot (immunoblot) technique to examine the immunoglobulin class-specific antibody response to individual *B. pertussis* antigens, both during an infection and as a result of vaccination.

This ELISA clearly has considerable potential for the rapid diagnosis of *B. pertussis* infection. An encouragingly high proportion of positive cases was detected by testing a single serum specimen collected at the time of presentation or admission. Under optimal circumstances, a result would be available on the day the serum was collected. A culture result, on the other hand, would not be available for at least 3 to 4 days. Culture of nasopharyngeal secretions was, however, essential, particularly in children less than 4 months old who had poor serologic responses in the present study (of 13 culture-proven cases, only 3 had positive ELISA results for serum specimens collected at the same time as the cultured nasopharyngeal secretions). In older children (>4 months), 35 of 45 culture-proven cases yielded positive results from serum specimens collected on the same day as the nasopharyngeal secretions. Trends similar to this have been reported in other studies (6). It has been known for many years that the probability of successfully isolating *B. pertussis* is greatest in the early stages of infection, at which time a serologic response may not yet be detectable. Thus, the two techniques (ELISA and culture) should be seen as complementary. Notwithstanding the above, the ELISA used in this study is highly sensitive, simple to perform, and, in most cases, the most rapid means of diagnosing pertussis.

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