Evaluation of Culture, Immunofluorescence, and Serology for the Diagnosis of Pertussis

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Nasopharyngeal culture, direct immunofluorescence, and serology of acute-phase and paired serum specimens were compared for the laboratory diagnosis of infections due to Bordetella pertussis in a community-based pediatric population with both high vaccine usage and high pertussis incidence. In 77 (37%) of 210 patients evaluated, one or more tests were positive for pertussis. A clinical illness compatible with pertussis was present in 52 (71%) of 73 pertussis test-positive and 42 (35%) of 119 test-negative patients (P < 0.001). Nasopharyngeal culture was of low sensitivity (20 [26%] of 77 positive tests) but was most commonly confirmed by another positive pertussis test (85%). Direct immunofluorescence was both insensitive and nonspecific; only 6 (30%) of 20 cases positive by culture were positive by immunofluorescence, and only 4 (33%) of 12 of the culture-negative, immunofluorescence-positive cases could be confirmed by another positive pertussis test. Although serology by enzyme immunoassay proved to be the most sensitive of the laboratory tests (87%), this sensitivity could be achieved only by assaying both acute-phase and paired serum specimens and measuring immunoglobulin G (IgG), IgA, and IgM antibodies to two pertussis antigens (pertussis toxin and filamentous hemagglutinin). Loss of sensitivity occurred with any reduction in the number of these serologic assays performed. Optimal laboratory diagnosis of endemic pertussis in a pediatric population requires both nasopharyngeal culture and serology by enzyme immunoassay.

The unavailability of sensitive laboratory tests for the diagnosis of pertussis hampers investigators who attempt to evaluate the epidemiology of the disease or the efficacy of the vaccine in preventing illness. Techniques routinely available in clinical laboratories include culture and direct immunofluorescence on nasopharyngeal secretions (2, 30); however, both tests lack sensitivity, and the latter also lacks specificity (3, 31). Some investigators have recently suggested that serologic methods may increase the diagnostic sensitivity. Such tests have usually measured specific antibody to the whole organism, Bordetella pertussis, in nasopharyngeal secretions (10) or in serum (21, 27, 39). More recently, these assays have been modified to detect antibodies to purported B. pertussis virulence factors (filamentous hemagglutinin [FHA] and pertussis toxin [PT]) in serum (1, 13, 15, 38) and in secretions (11). Unfortunately, the lack of standardized methods has prevented these tests from becoming universally available.

Difficulties in a prompt laboratory diagnosis of pertussis derive from the nature of the pathophysiology of the infection caused by B. pertussis. Typical signs and symptoms of pertussis do not occur until week 3 of infection, during the paroxysmal phase of illness (33). However, isolation of B. pertussis is most consistent in the catarrhal or early paroxysmal stage, often before the diagnosis of pertussis is suspected (24). In addition, infants, older children, adults, and previously immunized individuals may never develop the classical clinical manifestations of whooping cough. Although diagnostic methods relying on paired serum specimens (PS) may be more sensitive, they do not permit confirmation of infection during the acute phase, since proper timing of the follow-up serum is critical (31). Serologic methods using a single acute-phase serum specimen (AS) have also been proposed; these rely on combinations of titers of various antibody classes to differentiate antibody due to acute infection from antibody due to prior immunization (39).

Improved laboratory methods to aid in the diagnosis of pertussis are crucial in obtaining accurate epidemiologic data on pertussis incidence and vaccine efficacy. Recent studies have evaluated pertussis diagnostic tests which use crude bacterial antigen preparations (21), for diagnosis in unimmunized populations (39), or for diagnosis in adults during an outbreak (38). Although the results of these studies are generally favorable toward the tests, it is not clear whether these methods would be suitable for laboratory diagnosis of pertussis in an immunized population or during nonoutbreak situations. In this study we assessed a number of assays, including nasopharyngeal culture, direct immunofluorescence, and antibody to PT and FHA in AS and PS, in order to determine their suitability as diagnostic tests. The study was done in a community-based pediatric population with both high vaccine usage and high pertussis incidence.

MATERIALS AND METHODS

Population, specimen collection, and processing. The Izaak Walton Killam Hospital for Children (IWK) is the only pediatric hospital and diagnostic center for the city of Halifax (population, 250,000) and serves as a referral center for the maritime provinces of Canada (population, 1,500,000). The incidence of reported pertussis is approximately 13 cases per 100,000 people. Patients in whom pertussis was suspected were evaluated by nasopharyngeal culture and smear and by serology. At the time of presentation, secretions were collected by syringe aspiration through a fine flexible plastic catheter passed through the nose into the nasopharynx as previously described (10). In addition, a blood sample was obtained by finger stick, and a questionnaire detailing symptomatology, immunization status, and recent use of antimicrobial agents was completed.

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Arrangements were made for collection of a 3-week follow-up serum specimen; patients were reminded by telephone prior to their appointment. Nasopharyngeal secretions were immediately transported in the catheter from the Outpatient Diagnostic Centre to the microbiology laboratory of the IWK. There, nasopharyngeal secretions were expelled onto agar plates for culture and onto two clean glass slides for immunofluorescence. Smears were processed by treatment with aprotinin (4 U/ml) (18) and were stored at 4°C until examination. Serum was separated and stored at −20°C. Most specimens were completely processed within 1 h of collection, thereby eliminating the need for transport media; the few specimens collected overnight were held at 4°C prior to processing.

Control sera. A single serum specimen was collected from each of 210 children with no symptoms of respiratory illness who were admitted to the hospital for elective minor surgery. The control group included 88 children (42%) undergoing either insertion or removal of tympanostomy tubes, 52 (25%) having dental extractions or restorations, 14 (7%) undergoing circumcision or repair of circumcision, 13 (6%) undergoing herniorrhaphy, 13 (6%) undergoing superficial cyst removal or other minor plastic surgery procedures, and 23 (11%) undergoing other miscellaneous procedures (orchiopexy, cystoscopy, strabismus repair, etc.). The mean age was 6.5 years, with a range of 2 months to 15.9 years; 44% were less than 5 years of age. The immunization status of these children was not determined; however, immunization rates for the community were 94.8% in children under 5 years and increased to 98.3% after school entry (unpublished statistics, Nova Scotia Department of Health and Fitness).

Nasopharyngeal culture. Nasopharyngeal secretions were cultured on charcoal agar CM 119 (Oxoid Ltd., London, England) supplemented with 5% defibrinated horse blood and 40 μg of cephalixin per ml (35). Secretions were expelled directly onto two charcoal plates, one with and one without the cephalixin supplement. Plates were incubated at 36°C in a humid environment and were examined daily for 7 days for colonies typical of B. pertussis. Colonies with typical morphology were examined by Gram stain, and organisms with characteristic gram-negative morphology were confirmed by agglutination with B. pertussis antiserum and by absence of agglutination with B. parapertussis antiserum (Wellcome Research Laboratories, Beckenham, England).

Immunofluorescence. Direct smears of nasopharyngeal secretions were examined by standard methods (32). Slides were heat fixed and stained with either fluorescein-isothiocyanate-conjugated B. pertussis or B. parapertussis antiserum (Difco Laboratories, Detroit, Mich.). Positive and negative control slides were included in all assays; a smear was considered positive if three or more organisms with bright fluorescence-and typical morphology were observed on the slide stained with B. pertussis antiserum in the absence of fluorescence on the B. parapertussis-stained slides. The single observer who read all the slides was unaware of whether the smear was from a patient or was a positive control.

Enzyme immunoassay. Antibodies to PT and FHA in AS and PS were measured by enzyme immunoassay. PT, purified by the method of Sekura et al. (37), and FHA, purified by the method of Sato et al. (36), were adsorbed under alkaline conditions to duplicate wells of polystyrene microdilution plates (Nunc, Roskilde, Denmark) at concentrations of 5 μg/ml for 2 h at 37°C and then at 4°C overnight. Following a series of washes in phosphate-buffered saline with 0.05% Tween 20 (Sigma Chemical Co., St. Louis, Mo.) (PBS-T), remaining binding sites were blocked by a 1-h incubation at 37°C with PBS-T–10% normal goat serum (Woodlyn Laboratories, Guelph, Ontario, Canada). Following an incubation at 37°C of 1 h (for immunoglobulin G [IgG] and IgA assays) or 24 h (for IgM assays) and another series of washes, 100 μl of a 1:100 dilution of serum to be tested was added to duplicate wells. After an incubation at 37°C of 1 h (for immunoglobulin G [IgG] and IgA assays) or 24 h (for IgM assays) and another series of washes, 100 μl of peroxidase-conjugated goat F(ab') fragment antibody to either human IgG (Fc specific; dilution, 1:20,000), IgA (α specific; dilution, 1:4,000) or IgM (μ specific; dilution, 1:1,000) (all from Organon Teknika, Malvern, Pa.) was added to the wells, and they were incubated at 37°C for 1 h. Following a final series of washes, substrate consisting of o-phenylenediamine and hydrogen peroxide in a citrate-phosphate buffer was added to the plates. After incubation for 30 min at room temperature (≈22°C), the reaction was stopped with 100 μl of 4 N sulfuric acid and the A_{492} was determined in a BioTek 309 microelisa reader (Biotek Corp., Winooski, Vt.). Sera with high IgG, IgA, or IgM titers to PT or FHA, obtained from children with natural pertussis infection (IWK positive control sera), were serially diluted and run with each assay.

The enzyme-linked immunosorbent assay titer was defined as the reciprocal of the serum dilution that resulted in an A_{492} of 0.200. The titer was derived from the equation for the straight line described by the IWK positive control serum and the absorbance of the test serum at a dilution of 1:100 (16, 17). Standard sera, kindly provided by Charles Manclark at the Center for Drugs and Biologics, Food and Drug Administration, Bethesda, Md. (U.S. reference pertussis antiserum, human, lot 3), were assayed and compared with the IWK positive control sera used for this study (Table 1).

In PS, a fourfold rise in IgG or IgA or a fourfold rise or fall in IgM to PT or FHA was interpreted as a positive test. Only IgA and IgM were evaluated in single serum specimens; samples exceeding the mean of the control group by 2 standard deviations or more were interpreted as positive (Table 2). A patient was considered to have a serologic diagnosis of pertussis when one or more of the following conditions were met: (i) fourfold rise in IgG titer to PT or FHA, (ii) fourfold rise in IgA titer to PT or FHA, (iii) fourfold rise or fall in IgM titer to PT or FHA, or (iv) positive IgM and IgA titer in AS.

<table>
<thead>
<tr>
<th>TABLE 1. Comparison of IWK positive control sera with U.S. reference pertussis antiserum human lot 3</th>
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<tbody>
<tr>
<td>Antigen specificity</td>
</tr>
<tr>
<td>PT</td>
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<td></td>
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<td></td>
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<tr>
<td>FHA</td>
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</table>

* As calculated in Materials and Methods.

Table 1. Comparison of IWK positive control sera with U.S. reference pertussis antiserum human lot 3

Enzyme-linked immunosorbent assay (ELISA) as calculated by Manclark et al. (25).

Data analysis and ethical considerations. Sensitivity, specificity, and positive and negative predictive values were determined by standard methods (14, 34). False-positive and
false-negative rates can be calculated by subtracting specificity and sensitivity values, respectively, from 100 (14); these values are not included in the text. Differences in proportions were tested by chi square; Yates' correction was applied for contingency tables with 1 df. Student's t test was used for statistical analysis of the differences in antibody response between groups of patients. All statistical calculations were performed with a computer-programmed statistics package (StatSoft; Human Systems Dynamics, North Ridge, Calif.) on an Apple IIC personal computer (Apple Computers, Cupertino, Calif.); P ≤ 0.05 was considered statistically significant.

The protocol for this study was approved by the ethical review committee of the IWK.

RESULTS

Between November 1986 and December 1987, specimens from 502 patients were submitted for the diagnosis of pertussis. In 210 cases (42%), specimens included a nasopharyngeal aspirate for culture (NPAC) and smear direct fluorescent-antibody assay (DFA) and at least one serum sample. This subgroup constituted the study population. In 150 (71%) of 210 cases, PS was available. In 77 (37%) of the 210 patients, one or more tests were positive for pertussis. Culture was positive in 20 patients (9.5%), and immunofluorescence was positive in 18 (8.6%). A serologic diagnosis could be made in 67 patients, i.e., in 25 (12%) of 210 AS and 48 (32%) of 150 cases in which PS was available (this included 6 specimens also meeting the diagnostic criteria for AS).

Culture and immunofluorescence. Of the 77 pertussis test-positive patients, 20 (26%) were positive by NPAC and 18 (23%) were positive by DFA. Six patients were positive by both NPAC and DFA. A total of 17 (85%) of 20 NPAC-positive patients and 10 (56%) of 18 DFA-positive patients were also positive by serologic criteria. A total of 13 (93%) of 14 NPAC-positive DFA-negative cases and 4 (33%) of 12 NPAC-negative DFA-positive cases were also positive by serologic criteria. Assuming that culture positivity and a fourfold antibody rise are indicative of true-positives, NPAC had a sensitivity of 37%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 76%. DFA had values of 18, 93, 56, and 69%, respectively. By the less stringent criteria for true positivity of positive NPAC, DFA, PS, or AS, culture had a sensitivity of 26%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 60%; DFA had values of 22, 100, and 58%, respectively.

Serology. Mean IgG, IgA, and IgM antibody titers to PT and FHA were higher in the test-positive group than in the test-negative group (Table 3; P < 0.05 for all comparisons). Mean antibody titers in the test-negative group did not differ statistically from those that had been assessed in the control group (IgA and IgM to PT and FHA; Table 2).

AS. AS was available from all 210 patients; for 60 patients, this was the only serum specimen. A total of 25 (12%) of the patients had high IgM and IgA titers to PT or FHA in their first serum specimen; this accounted for 32% (25 of 77) of the positive patients. Only four patients positive by AS had a positive NPAC, suggesting that the organism may disappear coincidentally with antibody appearance. Sensitivity and specificity was 14% for single-specimen serology when stringent true-positive criteria were used and 31% by the less stringent definition. Specificity and positive and negative predictive values were 85 and 100%, 32 and 100%, and 66 and 61%, respectively, for stringent and less stringent criteria. The use of acute-phase serology detected 65% of positive patients when combined with NPAC and 76% when combined with NPAC and DFA.

PS. Two serum samples were available from each of 150 patients; 48 (32%) demonstrated a fourfold rise in IgG, IgA, or IgM titer to PT or FHA or a fourfold decrease in IgM. PS serology was the laboratory test which most often contributed to a positive result (67% of those positive); 17 (35%) of 48 patients positive by PS serology also had a positive response.
NPAC. When either definition of true positivity (see above) is used, sensitivity (94 or 67%), specificity (100%), positive predictive value (100%), and negative predictive value (97 or 76%) all compared favorably with values of the other diagnostic tests evaluated.

Of the 48 patients with serologic evidence in PS of infection, 34 had a rise in antibody to PT and 27 had a rise in antibody to FHA. An IgG rise was demonstrated in 28, an IgA rise was demonstrated in 26, and an IgM rise or fall was demonstrated in 23 patients. No antibody class or pertussis antigen alone was adequate to assess infection by PS serology. Measurement of a single antibody class to a single antigen detected 21 to 44% of the total seropositive cases (Table 4); the use of two antigens increased this to as high as 58%. Measuring two antibody classes to a single antigen detected 38 to 67% of positive cases, while measuring two antibody classes to two antigens made possible a detection rate of 88%. An additional six cases (12%) were detected only by measurement of IgG, IgA, and IgM to both PT and FHA.

The data were also assessed excluding the fluorescent antibody results. Of the 66 specimens positive by NPAC, or by PS or AS serology, 19 (29%) were positive by NPAC, 48 (73%) were positive by PS serology, and 22 (33%) were positive by AS serology. NPAC and PS serology together identified 51 (77%); NPAC and AS serology detected 37 (56%). Use of serologic PS or AS criteria without culture yielded 63 (95%) positives.

**Clinical data.** Clinical information was available for analysis on 192 patients; 119 patients were negative by all diagnostic tests, and 73 patients were positive by one or more assays.

(i) **Age.** The mean age of patients with a positive pertussis test was 4.9 years; the mean for patients who tested negative was 3.6 years (P = 0.01). There was no statistically significant difference in age of patients positive by different tests (Table 5).

(ii) **Immunization.** Both pertussis test-positive and test-negative patients had received a mean of 3.6 doses of pertussis vaccine prior to onset of illness (P = 0.5). The number of pertussis immunizations received by children positive by the various diagnostic tests ranged from 3.2 to 4.0 (Table 5); the differences were not statistically significant. A total of 17 (13%) of 133 test-negative patients and 14 (19%) of 73 test-positive patients had received fewer than three immunizations (P = 0.3); half of these patients were under 6 months of age.

Immunity to *B. pertussis* following vaccination decreases with the interval since immunization, with no demonstrable protection by 12 years (20). A total of 78% of the patients in this study had received a pertussis vaccination in the previous 2 years. Patients with negative pertussis tests had a mean interval since the most recent pertussis vaccine of 11.8 months. Patients with positive tests tended to have a longer interval since the last immunization (18.1 months) (Table 5); however, this reached statistical significance only in the group positive by AS serology criteria (22.1 months; P = 0.04).

(iii) **Clinical illness.** A total of 52 (71%) of 73 of pertussis test-positive patients and 42 (35%) of 119 of pertussis test-negative patients had an illness characteristic of pertussis (cough for more than 2 weeks, with vomiting, cyanosis, paroxysms, or apnea) (P < 0.001). A clinically compatible illness was present in significantly more patients positive by each test than in test-negative patients (Table 5) (P = 0.006). There was no difference demonstrated among the various tests. Duration of symptoms tended to be longer in test-positive patients but reached statistical significance only in patients positive by NPAC (34.1 versus 21.5 days; P = 0.02) and AS serology criteria (32.3 versus 21.5 days; P = 0.04).

(iv) **Antibiotic use.** A total of 63 patients (33%) were receiving antibiotics at the time of specimen collection; 37 (19%) of 192 were receiving an erythromycin-containing preparation. Three of these patients had a positive NPAC. No significant difference in antibiotic use was found between pertussis test-positive and test-negative patients (Table 5).

**DISCUSSION**

Evaluation of pertussis diagnostic tests is made difficult because there is no sensitive and consistently positive test (a

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**TABLE 4.** Sensitivity of antibody-class measurements in paired sera for the diagnosis of pertussis

<table>
<thead>
<tr>
<th>Antigen(s)</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgG + IgA</th>
<th>IgG + IgM</th>
<th>IgA + IgM</th>
<th>IgG + IgA + IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>42</td>
<td>44</td>
<td>33</td>
<td>52</td>
<td>63</td>
<td>67</td>
<td>73</td>
</tr>
<tr>
<td>FHA</td>
<td>29</td>
<td>21</td>
<td>27</td>
<td>38</td>
<td>48</td>
<td>46</td>
<td>56</td>
</tr>
<tr>
<td>PT and FHA</td>
<td>58</td>
<td>54</td>
<td>48</td>
<td>73</td>
<td>85</td>
<td>88</td>
<td>100</td>
</tr>
</tbody>
</table>

**TABLE 5.** Clinical data on 192 patients whose specimens were submitted for diagnosis of pertussis

<table>
<thead>
<tr>
<th>Laboratory test status</th>
<th>Patients</th>
<th>Mean age (yr)</th>
<th>Mean no. received</th>
<th>Mean interval since last dose (mo)</th>
<th>No. (%) meeting case definition&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean interval since onset (days)</th>
<th>No. (%) receiving erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>119</td>
<td>3.6</td>
<td>3.6</td>
<td>11.8</td>
<td>42 (35)</td>
<td>21.5</td>
<td>21 (18)</td>
</tr>
<tr>
<td>Positive</td>
<td>73</td>
<td>4.9</td>
<td>3.6</td>
<td>18.1</td>
<td>52 (71)</td>
<td>26.6</td>
<td>16 (22)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.4</td>
<td>3.5</td>
<td>19.2</td>
<td>16 (80)</td>
<td>34.1</td>
<td>3 (15)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>4.8</td>
<td>3.8</td>
<td>20.6</td>
<td>13 (72)</td>
<td>23.0</td>
<td>6 (33)</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>4.3</td>
<td>3.2</td>
<td>16.4</td>
<td>32 (68)</td>
<td>26.0</td>
<td>8 (17)</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>5.9</td>
<td>4.0</td>
<td>22.1</td>
<td>20 (87)</td>
<td>32.3</td>
<td>4 (17)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cough longer than 1 week, with paroxysms, vomiting, apnea, or cyanosis.
so-called "gold standard") against which other tests can be assessed. The use of multiple diagnostic tests in combination may result in improved sensitivity, but often with a concurrent loss of specificity (14). Additionally, evaluation of diagnostic tests in an outbreak situation can mask tests of low specificity, since the true positive rate is so uncommonly high. In this study we assessed this issue by using a combination of tests as potential "gold standards," and these were then compared with single or multiple combinations of tests. The study was performed during a 14-month period of moderate endemic pertussis activity rather than during an outbreak. During this period, 20 culture-confirmed cases occurred; by contrast, 88 culture-confirmed cases had occurred during an epidemic year encompassed in the preceding 14-month period (unpublished data).

Nasopharyngeal culture proved to be a test of low sensitivity but high specificity, while direct immunofluorescence lacked both sensitivity and specificity. These results of our assessment of methods for detecting bacteria in nasopharyngeal secretions are similar to those in previous reports. By a variety of criteria, culture sensitivity has been estimated to be 30 to 60% (6, 12, 13, 15, 27, 28, 38, 39; S. A. Young, G. L. Anderson, and P. D. Mitchell, Clin. Microbiol. Newsl. 9: 176–179, 1987), while direct immunofluorescence has been reported to be positive in 20 to 100% of culture-positive cases (5, 7, 9, 19, 26; Young et al., Clin. Microbiol. Newsl.). Our apparent false-positive rate of 44% (those positive by DFA only) is higher than that in other reports (0 to 40%) (5, 8, 9, 19; Young et al., Clin. Microbiol. Newsl.) and may be attributable to a number of factors. Most important is the actual definition of false positivity, since our study required another positive laboratory test to confirm the DFA results, whereas other researchers often used a compatible clinical illness as sufficient confirmation. Observer inexperience and variability have also been linked to the difficulties with the DFA assay (3); however, the use of a single experienced observer in this study should have decreased this type of error. Prior use of antimicrobial agents has often been advanced to explain DFA-positive culture-negative specimens (31; Young et al., Clin. Microbiol. Newsl.). Indeed, a higher proportion of DFA-positive culture-negative patients (5 [51%] of 10) than of DFA-positive culture-positive patients (11 [17%] of 69) were receiving erythromycin. These results did not reach statistical significance.

Antibody determinations in PS proved to be the most sensitive of the laboratory tests; however, no single antibody or antigen test was sufficient to consistently confirm the diagnosis. Although optimal results were achieved by using two antigens and measuring three antibody classes, it is possible that additional cases could have been detected by using additional antigens or by increasing the interval between the two serum specimens. The ideal serologic test would be available for a single specimen at the time of patient presentation and would differentiate between natural infection and immunity derived through immunization. Following infection or vaccination, both IgG and IgM antibodies are formed; IgA is elicited only following infection (4, 22, 23, 27, 29, 39). Since IgG antibodies are long lived, a rising titer in PS is required for the diagnosis of pertussis. In contrast, IgM antibodies have a short half-life, and their presence indicates recent exposure to pertussis antigens. IgA may remain elevated for 6 months to 2 years (23, 29). Thus, the presence in a single serum of both IgA and IgM suggests a recent natural infection.

The single-serum test results suffered from a lack of sensitivity and specificity, since positive samples frequently were not confirmed by other tests. It is possible that the serologic cutoff titers used for IgM and IgA were too low and that children with high titers from previous immunization or illness were included. This is unlikely, since only titers greater than 2 standard deviations above those of a comparably aged population were considered positive and since positivity was limited to individuals exceeding those values for both IgM and IgA. None of the 210 healthy control children met these criteria for infection. Similarly, the erroneous inclusion of children with persistent high IgA from a previous natural infection and high IgM from a recent immunization is unlikely, since children with previous infection would more likely have an anamnestic IgG rather than IgM rise (12). Interestingly, the interval since a previous pertussis vaccine dose was longest in children positive by AS criteria. It is more likely that our criteria for seropositivity on single specimens were too restrictive, particularly since the control children came from the community in which there is a high rate of endemic pertussis infection. However, these criteria have previously correlated well with clinical pertussis (38, 39); in our study, over 91% of patients positive by AS serology criteria had a compatible illness.

It is not surprising that there was poor correlation between patients testing positive by culture and PS serology and those testing positive by AS serology. Nasopharyngeal cultures for pertussis are uniformly positive early in the illness and become negative during the paroxysmal stage (24, 31, 33). Similarly, patients demonstrating a rise in antibody to pertussis antigens would likely have been seen early in their illnesses, at the time the first serum specimen was taken. In contrast, patients in whom high titers are already present at the time of first sampling would tend to be later in their course, when cultures are consistently negative. Indeed, the patients positive by AS serology criteria had the longest interval since onset of symptoms.

The results of this study corroborate and extend the work of previous investigators with certain exceptions. In our study, DFA was difficult to interpret and of low specificity and may be best suited for areas where there is a high pertussis prevalence or for an outbreak situation. Like other investigators (12, 15, 39), we found that serologic methods were the most sensitive and specific but that a combination of assays was required for optimal results. Our data support the findings of Steketee et al. (38), which demonstrated the utility of the enzyme immunoassay with purified pertussis antigens for the diagnosis of pertussis during an outbreak among institutionalized adults, and we extend these observations to include endemic community-based illness in a pediatric population. In contrast to their study, ours was unable in most cases to correlate the type of pertussis test-positive case with the duration of illness. However, more-accurate clinical data from a closed adult residential facility were probably available in their population and may explain these differences.

Pertussis continues to be an elusive infection in terms of its immunity, diagnosis, and control through immunization. Our report documents the continued need for improvements in diagnostic tests for pertussis and demonstrates that optimal laboratory diagnosis currently requires both nasopharyngeal culture and serology by enzyme immunoassay. Epidemiologic data based only on typical clinical presentation and culture are likely to underestimate the true incidence of pertussis in a community.
EVALUATION OF METHODS FOR THE DIAGNOSIS OF PERTUSSIS

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LITERATURE CITED


