Specific Immunoglobulin A to *Bordetella pertussis* Antigens in Mucosal Secretion for Rapid Diagnosis of Whooping Cough

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Specific immunoglobulin A (IgA) to *Bordetella pertussis* filamentous hemagglutinin (FHA) and pertussis toxin (PT) was determined in mucosal secretions by an enzyme-linked immunosorbent assay (ELISA). It took 3 to 4 h to complete the ELISA. The upper limits of normal values for age were determined in nasopharyngeal (NPH) secretions from 23 patients with viral infections and in 10 healthy adults working with pertussis patients or cultures. A significant IgA response to FHA was found in 38 of 54 (70%) and to PT in 28 of 54 (52%) NPH secretions from patients with pertussis confirmed by culture, serology, or both. The rate of positive responses to either antigen (44 of 54 [81%]) was significantly higher than that by culture alone (29 of 54 [54%]; \(P < 0.01\)). The rate of positive responses increased from 65% in patients with symptoms for 1 week or less to 87 to 92% in patients with symptoms for 2 or more weeks. The specific IgA response to PT was found in 100% of NPH samples from 17 unimmunized children less than 3 years of age and in only 30% of adults and immunized children greater than 3 years of age. A response to FHA was found in 65 to 73% of the NPH secretions in all age groups. Saliva samples were found to contain specific IgA to FHA and PT in all age groups, but these were of diagnostic value in 50% (11 of 22) of the adult patients. The specificity of the ELISA was 100% (10 of 10 negatives) in NPH secretions from patients with pertussis-like cough who had negative cultures and serology. The results indicate that determination of specific IgA to PT and FHA in NPH aspirates represents a sensitive and rapid diagnostic method for the detection of pertussis.

Culture of *Bordetella pertussis* is the method most commonly used for the diagnosis of whooping cough. Although highly specific, the sensitivity of culture has repeatedly been shown to be low (5, 7). The slow growth of the bacteria is another disadvantage, requiring 3 to 5 days of incubation for isolation from clinical specimens.

The sensitivity of pertussis diagnostic methods can be greatly improved by use of serologic assays as a complement to the culture method (5, 6, 9). These assays rely on the development of a systemic antibody response, which delays the diagnosis of the disease. The rapid diagnostic method of direct immunofluorescence can be used, but it has several drawbacks. The sensitivity of direct immunofluorescence is similar to that of culture under optimal conditions (1, 6, 10).

The specificity has been questioned, mainly due to poor commercial reagents and because of the subjective evaluation requiring trained microscopists for satisfactory results (1, 10).

In a study by Goodman et al. (3), it was suggest that detection of specific immunoglobulin A (IgA) to *B. pertussis* in nasopharyngeal (NPH) secretions may be a useful diagnostic method. In a more recent study encouraging results have been shown (13) with respect to this method. The use of more than one highly purified bacterial antigen in assays of *B. pertussis* antibody could, in theory, increase the sensitivity. Purified antigens would also diminish the risk of false-positive results.

In previous studies NPH aspirates were used as samples. This test material has the advantage that specific IgA determination can be combined with tests for viral antigens with differential diagnostic significance. The possibility that other specimens, such as saliva, could be of diagnostic value has not been evaluated. Although it has been shown in several studies that NPH swabs yield more positive cultures for *B. pertussis* than the old cough plate, the presence of specific IgA to *B. pertussis* antigens in saliva has, to our knowledge, not been investigated.

The aim of the present study was to develop and standardize an enzyme-linked immunosorbent assay (ELISA) for the rapid determination of specific IgA to the filamentous hemagglutinin (FHA) and pertussis toxin (PT). The method was evaluated in NPH secretions and saliva from patients with pertussis or viral infections and healthy adults.

**MATERIALS AND METHODS**

**Patient material.** One hundred patients with respiratory symptoms were enrolled into the study, which was conducted between October 1982 and March 1983. The suspicion of pertussis was based on clinical or epidemiological grounds. An NPH aspirate and blood sample (serology sample 1) were taken from all patients on the first visit. During a second visit made by 51 patients a mean of 60 days (range, 27 to 129 days) after the onset of disease, serology sample 2 was obtained. A second NPH aspirate was obtained during the same visit. Saliva samples were collected during both visits from patients willing to undergo sampling. Patients (n = 33) without a serology sample 2 were excluded from the study if the disease was not confirmed by culture. Three additional patients were excluded; one because of an inadequate sampling interval between the times that the two blood samples were obtained and two patients because of uncertain serology results. The final material from patients came from 54 patients with pertussis confirmed by culture alone (n = 16), by both culture and serology (n = 13), and by serology alone (n = 25).

The 17 children under 3 years of age who took part in this...
study were not immunized against pertussis, while the 15 children 4 to 15 years of age who took part in this study had all received three of the diphertheria, pertussis, and tetanus vaccine as infants. Patients \( n = 10 \) with respiratory symptoms possibly caused by \( B. \) pertussis but negative both by culture and serology served as a disease control group. Other nonpertussis patient controls consisted of children with respiratory syncytial virus infection confirmed by immuno-fluorescence \( n = 17 \) and adults with influenza A virus infection confirmed by immuno-fluorescence \( n = 6 \). NPH aspirates \( n = 10 \) and saliva \( n = 17 \) from healthy hospital and laboratory staff in contact with pertussis patients or samples from patients with pertussis but without respiratory symptoms were used to establish normal levels of \( B. \) pertussis antibody.

**Sampling of patients and handling of samples.** For adults blood samples were drawn by venous sampling, and for children blood samples were obtained by capillary sampling in Microtainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, N.J.). The serum samples were kept at \(-20^\circ C\) until the paired sera were analyzed in parallel.

NPH aspirates were obtained by introducing a fine plastic tubing (baby feeding tube 4; diameter, 1.32 mm; Vycom, France) into the nasopharynx and applying suction with a pressure of \( 2 \mathrm{~kg/cm}^2 \). The content of the plastic tubing was flushed out with approximately 0.5 ml of sterile saline. No attempts were made to further standardize sampling conditions or sample volumes. The study design was selected to represent a usual clinical situation. The nurses were therefore only instructed to follow the routine procedures for obtaining aspirates taken for the rapid diagnosis of viral infections. For the saliva samples, the patients were asked to cough strongly and then to deliver the saliva-sputum into a sterile plastic container.

The NPH aspirates were obtained during the first visit from 64 subjects and during the second visit from 29 subjects. Saliva samples were collected during the first visit from 45 patients and during the second visit from 22 patients. Each sample was diluted with an equal amount of a 20\% (wt/vol) solution of bovine serum albumin (BSA) in a phosphate-buffered saline (PBS) before it was frozen at \(-20^\circ C\).

**Culture of \( B. \) pertussis.** NPH aspirates and saliva samples were streaked with 10-\( \mu \)l disposable inoculation loops (Nunc, Roskilde, Denmark) on Bordet-Gengou agar plates supplemented with 10\% horse blood. The plates were incubated for 5 days at \(37^\circ C\). Isolates were verified as \( B. \) pertussis by agglutination and immunofluorescence. Results of the cultures given here are based on isolates from NPH aspirates. Only one saliva sample was positive for \( B. \) pertussis, as was the NPH aspirate obtained from the same patient during the same visit.

**Antigens and reagents for ELISA.** Two antigens, FHA and PT, were used both for antibody determination in serum and in NPH and saliva samples. The FHA was purified from shaken cultures of \( B. \) pertussis 247/82 serotypes 1, 2, and 3 (National Bacteriology Laboratory [NBL], Stockholm, Sweden) essentially as described by Cowell et al. (2). Four liters of sterile-filtered culture supernatant, adjusted to pH 8.7, was passed through a column containing 100 ml of hydroxypapotite (HA-Ultroget; LKB, Bromma, Sweden) equilibrated with 0.01 M sodium phosphate (pH 8.0) at a rate of 500 ml/h. After the column was washed with 500 ml of 0.01 M sodium phosphate (pH 8.0)-500 ml of 0.1 M sodium phosphate (pH 8.0), the bound material (FHA) was eluted with 0.1 M sodium phosphate (pH 7.0) containing 0.5 M NaCl. Fractions of 5 ml were collected. FHA eluted as a single peak, and the material was pooled (about 200 ml).

Analysis by sodium dodecyl sulfate-gel electrophoresis showed the expected high-molecular-weight bands of FHA when the gels were stained with Commassie blue R-250 (Fig. 1A, lane 1). The FHA preparation in Fig. 1A, lane 2, kindly provided by Biken (Osaka, Japan), was used in some preliminary experiments (data not shown).

PT was kindly provided by the Research Foundation for Microbiological Diseases (Biken, Osaka University, Osaka, Japan). Sodium dodecyl sulfate-gel electrophoresis of the preparation is shown in Fig. 1B, lane 2, in which no bands but the five subunits of PT can be seen. For reference, a preparation from NBL was included (Fig. 1B, lane 1).

For determination of mucosal antibodies, a second coating layer of human serum albumin (HSA) was applied. A highly purified routine HSA preparation from NBL was used.

The enzyme conjugates used for serum antibody determinations were swine anti-human IgG, IgM, and IgA (lots MC1, LM3, and MC2, respectively; Orion Diagnostica, Espoo, Finland). Dilutions of 1:100, 1:50, and 1:25, respectively, were chosen by checkerboard titrations against negative and positive sera. For the mucosal IgA determination, anti-human IgG (Orion) was used at a 1:50 dilution. Other anti-human IgA conjugates investigated for the mucosal IgA determination were rabbit anti-human IgA (lot 0.75; DAKO-patts, Copenhagen, Denmark), goat anti-human IgA (lot E218; ICN Immuno Biologicals, Lisle, Ill.), and goat anti-human IgA (lot 36 F8990; Sigma Chemical Co., St. Louis, Mo.).

**ELISA for specific IgA and IgG determinations in mucosal secretions.** Coating of cobalt-irradiated 96-well polystyrene microplates (M129B; Dynatech, Plochingen, Federal Republic of Germany) with FHA (2 \( \mu \)g/ml) and PT (1 \( \mu \)g/ml) diluted in PBS (pH 7.2) was done at room temperature (22\(^\circ\)C) overnight. Volumes of 100 \( \mu \)l were used throughout the test. The plates were washed with physiological saline supplemented with 0.05\% Tween 20 four times between each step. The second coating layer of 2\% HSA in PBS was added, and the plates were incubated for 1 h at 22\(^\circ\)C. The plates thus coated were either used immediately or could be kept at \(-70^\circ C\) for at least 2 months. The NPH aspirates and saliva samples were screened at a final dilution of 1:100 (diluted 1:2 in BSA and 1:50 in PBS). Samples with \( A_{405} \) values of 0.8 or

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**FIG. 1.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of purified FHA (A) and PT (B). (A) Lane 1, FHA preparation from NBL; lane 2, FHA preparation from Biken. (B) Lane 1, PT preparation from NBL; lane 2, PT preparation from Biken.
higher were retested in threefold dilution steps. Samples with $A_{405}$ values of 0.2 or lower were retested at a final dilution of 1:20. The samples were incubated with antigens for 1 h at 22°C. All determinations were made in duplicate and were repeated if the variation coefficient was 10% or more. Incubation with the rabbit anti-human IgA (DAKO-patts) used throughout this study at a dilution of 1:150 was done for 1 h at 22°C. After the addition of the substrate p-nitrophenyl phosphate (Sigma), the $A_{405}$ values were determined when the positive and negative controls had reached certain predetermined values, i.e., after 20 to 30 min.

The ELISA for IgG determination was performed under the same conditions as the IgA assay by using a final 1:20 dilution of the specimens. The IgG conjugate (Orion) was used at a dilution of 1:50.

In Fig. 2 through 5, IgA titers are expressed in relation to the upper limit of normal values for age (cutoff level). The cutoff level established in healthy controls was set at 1, and the samples were adjusted accordingly.

ELISA for antibody determination in serum. The ELISA for antibody determination in serum was performed essentially as described previously (5). PT was used at a coating dose of 1 µg/ml, and FHA was used at 2 µg/ml. Serum samples were tested in duplicate by single-point determination at a dilution of 1:500. Higher dilutions were made, if necessary, to demonstrate increases in titer. Coating was done overnight at room temperature (22°C), and the plates were kept frozen at −70°C until use. Serum incubation was for 1 h at 22°C for IgG and IgA determinations and 2 h at 37°C for IgM. Incubation with the alkaline phosphate conjugates to human IgG, IgM, and IgA (Orion) was done at 22°C overnight. The substrate was added at 22°C, and the reading of the microplates was done in a Titertek Multiscan apparatus (Flow Laboratories, Irvine, Scotland), when the positive controls reached predetermined values (after 45 to 65 min).

A significant increase in titers was defined as at least a twofold increase between paired samples.

Neutralization test for PT. The neutralization test for PT was done as described previously (4). Briefly, twofold dilutions of patient serum were incubated with a predetermined four clustering units of PT before $10^6$ CHO cells were added to the mixture. The neutralization of the clustering effect of PT by antibodies in the samples was determined after incubation for 48 h. A significant increase in the titers was defined as at least a fourfold increase between two samples tested in parallel.

A patient positive by serology was defined as one who showed a significant rise in titers by IgG or IgA to FHA or to PT or both by ELISA or a significant rise by the neutralization test.

Statistical analysis. Differences between means were compared by Student's t test and differences between groups were compared by chi-square tabulation, with Yates correction when appropriate.

RESULTS

Optimal conditions for the mucosal ELISA and choice of cutoff levels. The four anti-human IgA conjugates were tested by checkerboard titrations, and two (DAKOpatts and Sigma) were found to give satisfactory results. Incubation times down to 15 min were tested. The low dilution of conjugates necessary for incubation times of under 30 min resulted in higher nonspecific background binding. Incubation for 60 min was chosen but could be reduced to 30 min. The incubation time for the samples was varied from 15 to 60 min; but slight losses of sensitivity were noted with decreasing times, and therefore, 60 min of incubation was used.

A second coating layer of BSA or HSA both decreased the nonspecific background. No difference was found with the patient samples supplemented with BSA before they were frozen. Such a supplement to the samples prior to freezing was not found to be necessary in later studies. A second coating with HSA was therefore chosen to avoid introduction of another species in the detection system.

The upper limit of normal $A_{405}$ values determined in the negative control samples was set at mean ± 3 standard deviations. For mucosal IgA to FHA, the cutoff levels were set at 0.07, 0.14, 0.21, and 0.3 in age groups of <3 years, 4 to 7 years, 8 to 15 years, and adults, respectively, by using 1:20 dilutions of the NPH aspirates and saliva samples. For secretory IgA to PT, the cutoff levels were 0.15, 0.48, and 0.77 in the age groups of <3 years, 4 to 15 years, and adults, respectively. One 12-month-old child with respiratory syncytial virus infection who was used as a control showed a high secretory IgA titer to PT. Scrutiny of the case history of the child disclosed that the child had a culture-confirmed B. pertussis infection 7 weeks before admission for the respiratory syncytial virus infection at the time of the sampling. This child was excluded from the calculation of the cutoff levels but was included in the data given in Fig. 2 and 3.

Specific IgA in NPH aspirates. Specific IgA to FHA was detected in a total of 38 of 54 (70%) and to PT in 28 of 54 (52%) NPH samples taken during the time of the first visit of the patients with pertussis (Table 1). A positive result to either FHA or PT was obtained in 44 of 54 (81%) patients. This was a significantly higher positive rate than that obtained by culture of B. pertussis (29 of 54 [54%]; $P < 0.01$). The IgA response to FHA showed little difference by age or immunization status. On the other hand, the specific IgA response to PT showed a marked variation, with 100% positive results in the youngest children and only about 30% in the older immunized children and in adults.

The distribution of specific IgA to either antigen was positive for 23 of 29 (79%) of the culture-confirmed cases and for 21 of 28 (84%) of the culture-negative cases (not significant). The mean disease duration in the culture-confirmed cases was 10.3 ± 7.1 days (range, 1 to 27 days), and in the
serology-confirmed cases it was 20.6 ± 9 days (range, 5 to 42 days; \( P < 0.001 \)). The relation of disease duration at the time of sampling to the sensitivity of the IgA assay is shown in Table 2. During the first week of disease, 65% of the patients were positive for either antigen. This rate increased to 87% during the second week and was 89 to 92% during the following weeks. The differences were not statistically significant.

The relation between the IgA responses to FHA and PT and the time after the onset of symptoms are given in Fig. 2 and 3. To show the kinetics of the IgA responses, Fig. 2 and 3 also include data for the second NPH samples taken at the second visit for the convalescent-phase blood samples. Adults and older children showed a much higher fold increase above cutoff levels to FHA than to PT in the first sample. IgA titers in these patients tended to decrease with time after the onset of disease, although two samples drawn as late as 90 days or more after the onset of disease were still weakly positive to FHA. In the unimmunized younger children, IgA levels showed increases over time both to PT and to FHA.

Fig. 2 and 3 also illustrate the specificity of the IgA assay. All 10 patients (12 samples) with negative culture and serology had negative NPH results to both FHA and PT.

**Specific IgA antibodies in saliva.** The results for specific IgA antibodies in saliva in patients with pertussis are shown in Table 3. The cutoff levels established in NPH samples were used for all age groups, as saliva samples from healthy children were not available. These cutoff levels gave negative results in 15 of 17 (88%) healthy adults and 3 of 4 patients with non-pertussis disease to FHA, while 17 of 17 and 4 of 4 of the saliva samples, respectively, were negative for IgA antibodies to PT (Fig. 4 and 5). The results indicate that the cutoff levels obtained in NPH aspirates were set too low for IgA to FHA but were adequate for IgA to PT. A higher cutoff level for IgA to FHA, e.g., set at 2 in Fig. 4, would increase the specificity to 17 of 17 healthy adults and 4 of 4 patients with non-pertussis, still leaving 11 of 22 (50%) positive adult patients with pertussis. Figures 4 and 5 also illustrate that there was a substantial IgA response to pertussis FHA in saliva from adults, while it was very low or nonexistent in younger, unimmunized children. The IgA to PT in saliva from the younger children was only pronounced in late samples. In adults, a response to PT was recorded in more saliva samples (55%) than in NPH secretions (32%), but the difference was not statistically significant (Tables 1 and 3).

**Specific IgG in mucosal secretions.** None of the youngest unimmunized children showed an IgG response to FHA and only 7 of 17 (41%) showed such a response to PT (Table 4). Older children showed more of an IgG response to FHA than of IgA (53 versus 27%; Tables 1 and 4) but less of an IgG response to FHA (33 versus 73%). Adults showed a tendency toward more of an IgG response (76%) than an IgA response (32%) to PT in NPH secretions, while the FHA response was lower. None of the differences were statistically significant.

The cutoff levels for IgG in NPH secretions to PH were established at 0.03 and 0.06 for children <3 years of age and all other groups, respectively. A common cutoff level of 0.19 was used for all age groups for IgG to FHA. With these limits, the 10 patients (12 samples) with non-pertussis disease were all negative. The IgG response in saliva from adults, with cutoff levels established in 17 healthy adult controls, was much weaker than the IgA response to both FHA and PT (Table 4).

**DISCUSSION**

This is the first investigation of the IgA and IgG responses in human mucosal secretions to purified *B. pertussis* FHA

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**TABLE 1.** Specific IgA determined by ELISA* in NPH aspirates from patients with pertussis confirmed by culture*, serology, or both

<table>
<thead>
<tr>
<th>Patients with pertussis</th>
<th>No.</th>
<th>No. (%) of specific IgA positive to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FHA</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-3 yr</td>
<td>17</td>
<td>11 (65)</td>
</tr>
<tr>
<td>4-15 yr</td>
<td>15</td>
<td>11 (73)</td>
</tr>
<tr>
<td>Adults</td>
<td>22</td>
<td>16 (73)</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>38 (70)</td>
</tr>
</tbody>
</table>

* ELISA was for 3 to 4 h.
* There were 29 of 54 (54%) positive results by culture.

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**TABLE 2.** Sensitivity of ELISA* for IgA antibodies to FHA and PT in relation to disease duration

<table>
<thead>
<tr>
<th>Time (days) after onset of disease</th>
<th>No.</th>
<th>No. (%) of specific IgA positive to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FHA</td>
</tr>
<tr>
<td>0-7</td>
<td>17</td>
<td>9 (53)</td>
</tr>
<tr>
<td>8-14</td>
<td>15</td>
<td>11 (73)</td>
</tr>
<tr>
<td>15-21</td>
<td>9</td>
<td>7 (78)</td>
</tr>
<tr>
<td>&gt; 21</td>
<td>13</td>
<td>11 (85)</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>38 (70)</td>
</tr>
</tbody>
</table>

* ELISA was for 3 to 4 h.

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**TABLE 3.** Specific IgA to FHA and PT in the first saliva samples with cutoff levels established in NPH aspirates*

<table>
<thead>
<tr>
<th>Patients with pertussis</th>
<th>No.</th>
<th>No. (%) of specific IgA positive to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FHA</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-3 yr</td>
<td>4</td>
<td>3 (75)</td>
</tr>
<tr>
<td>4-15 yr</td>
<td>13</td>
<td>7 (54)</td>
</tr>
<tr>
<td>Adults</td>
<td>22</td>
<td>17 (77)</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>27 (69)</td>
</tr>
</tbody>
</table>

* The specificity of the assay with these limits is shown in Fig. 4 and 5.

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**FIG. 3.** Specific IgA antibodies to PT in NPH aspirates from patients with pertussis and from controls. Symbols are as described in the legend to Fig. 2.
FIG. 4. Specific IgA antibodies to FHA in saliva from patients with pertussis and from controls, with the upper limit of normal values established in NPH aspirates as cutoff levels. Symbols are as described in the legend to Fig. 2.

and PT. The results indicated that determination of specific IgA to these antigens in NPH secretions represents a rapid diagnostic method for pertussis.

The finding of differences in the IgA response pattern in NPH secretions from patients of different ages was interesting. A higher IgA response to FHA in sera from adults compared with sera from infants has also been noted before (5). The marked difference in the IgA response to PT, with 100% positive responses in the youngest children versus some 30% in older children and adults, was unexpected. Whether the difference was due to the difference in age or in immunization status or a combination of both factors cannot be answered from the results of the present study. In Sweden, general immunization against pertussis was discontinued in 1979. All the children in the study born prior to the cessation were immunized, while all the younger children were unimmunized.

Determination of IgG antibodies was made to investigate the possibility that the differences in IgA response could be due to an antibody competition between IgA and IgG for binding sites in the ELISA. There were no indications of a substantial competition, as some of the NPH secretions with the highest IgG titers also had the highest IgA titers. Antibody determinations of both classes (IgA and IgG) could, in theory, increase the diagnostic sensitivity. Analysis of the data with this objective showed that two additional patients (immunized children aged 4 and 5 years) would have been diagnosed with pertussis by a positive IgG response to PT. It is questionable, however, whether such a small gain motivates the use of another conjugate and is worth the risk of decreased specificity with an additional determination.

The saliva samples from adults were found to contain high levels of specific IgA, IgG, or both to PT and FHA. As younger children were less willing or able to collaborate in the collection of saliva, the age group with good responses was the one most eligible for this type of sampling. Saliva may be easier to obtain under field conditions, e.g., in outbreak investigations, and could represent an alternative to NPH aspirates.

The antibody response in mucosal secretions raises interesting questions on the role of these antibodies in disease and for immunity. The very rapid IgA response to PT in NPH secretions from all the youngest children compared with the response in those from adults seems to have little influence on the course of the disease, since the youngest unimmunized children had a more severe, typical presentation of whooping cough. The lack of a demonstrable IgG response to FHA in NPH secretions from this age group in the natural disease raises another question concerning the relevance of this antigen for protection against pertussis disease, colonization, or both. Our earlier finding of a low and transient IgG response to FHA in serum pointed in the same direction (5). Antibody to FHA has been shown to protect mice against both intranasal and intracerebral challenge with B. pertussis (12). Results obtained in mice, a species that does not naturally acquire B. pertussis infection, may not necessarily be relevant for humans. The present data could therefore also contribute to the discussion on the role of FHA in acellular pertussis vaccines (11).

The value of IgA antibody determination in NPH secretions as a rapid diagnostic method was firmly established in this study. An overall sensitivity of 81% was obtained by determination of IgA to both FHA and PT, irrespective of disease duration prior to sampling. This rate should be compared with the sensitivity of 54% for culture (P < 0.01). The IgA determination, taking 3 to 4 h to complete, represents a major improvement over the 3 to 5 days necessary for culture. The sensitivity of the rapid ELISA for IgA in NPH samples increased with disease duration, from 65% during the first week to 92% after more than 3 weeks of disease.

The relative merits of IgA determination to PT or FHA and the diagnostic sensitivity cannot be generalized from the

FIG. 5. Specific IgA antibodies to PT in saliva from patients with pertussis and from controls, with the upper limit of normal values established in NPH aspirates as cutoff levels. Symbols are as described in the legend to Fig. 2.

### TABLE 4. Specific IgG antibodies to FHA and PT in the first 54 weeks of disease

<table>
<thead>
<tr>
<th>Patients with pertussis</th>
<th>No.</th>
<th>No. (%) of specific IgG positive to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FHA</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3 yr</td>
<td>17</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4–15 yr</td>
<td>15</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPH</td>
<td>21</td>
<td>12 (57)</td>
</tr>
<tr>
<td>Saliva</td>
<td>21</td>
<td>3 (14)</td>
</tr>
</tbody>
</table>

* Cutoff levels established in 17 healthy adult controls.
* ELISA was for 3 to 4 h.
results of this study. The samples were collected at the height of a major pertussis epidemic in 1982 and 1983 in Sweden, which was the largest epidemic since the 1960s. The possibility of pertussis in a coughing child was immediately suspected, leading to a short delay before the patient saw a doctor. In fact 10 to 32 (31%) of our pediatric patients had a disease duration of 7 days or less at the time of sampling and 19 of 32 (59%) had a disease duration of 14 days or less. Therefore, although the high sensitivity of IgA determination to PT in the youngest unimmunized children favorably influenced our results in Sweden, the short delay in suspecting the correct diagnosis decreased our sensitivity. Even a majority of our adult patients sought medical attention with a short disease duration (7 days or less in 6 of 22 [27%] and 14 days or less in 12 of 22 [55%] patients). Pertussis in adults, a major group at risk to acquire the disease upon the return of pertussis (15), was a well-recognized possibility for both the public and Swedish physicians. This awareness of pertussis as a possible cause of cough in all age groups is in contrast to the experience of countries with an efficient immunization program (8, 14). The delayed clinical suspicion of pertussis, especially in immunized children and adults, favorably influences the sensitivity of the assay in such a setting.

The increase in the rate of positive results and in the height of titers with disease duration in the IgA assay shows the opposite kinetics of the sensitivity of culture for B. pertussis. The IgA assay therefore also represents a valuable complement to culture. The same decrease in sensitivity with disease duration is seen in serology, i.e., in terms of positive serology based on titer rises. Patients seeking medical care after several weeks of disease tend to have high titers already in their acute sample. The interpretation of these serologic responses based on high titers is more difficult and can be less specific than diagnosis based on titer rises. Three patients with such a serologic response were excluded from the evaluation. For all three patients results were clear-cut and positive by the IgA test. The proportion of such cases in a population also increases in a setting with long delays by both patients and doctors. It is therefore likely that the combination of culture, specific IgA determination of FHA and PT in mucosal secretions, and serum antibody determination to the same antigens can result in a higher sensitivity than that achieved by any of the assays alone.

The specificity (100%) of the IgA assay in patients with possible pertussis excluded by culture and serology was highly satisfactory. The number of patients (n = 10) studied was small, however, due to losses in the follow-up with convalescent sera. The results obtained in this pilot study will be expanded in an ongoing large clinical study that includes several other rapid diagnostic methods.

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LITERATURE CITED