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Characterization of Serological Responses to Pertussis

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We have compared the use of five nonvaccine antigens to the use of conventional vaccine antigens, pertussis toxin (PT), and filamentous hemagglutinin (FHA) for the serological diagnosis of pertussis by enzyme-linked immunosorbent assay (ELISA). The nonvaccine antigens included the catalytic region of adenylate cyclase toxin (CatACT), the C-terminal region of FHA (C-FHA), lipooligosaccharide (LOS), the peptidoglycan-associated lipoprotein (PAL), and the BrkA protein. The serological responses of individuals with culture-confirmed pertussis were compared to those of adults with no recent history of a coughing disease. An immunoglobulin G (IgG) ELISA for PT was the most sensitive (92.2%) test for the serodiagnosis of pertussis. Of the nonvaccine antigens, ELISA for IgG responses to CatACT (sensitivity, 62.8%), C-FHA (sensitivity, 39.2%), and LOS IgA (sensitivity, 29.4%) were less sensitive but could also distinguish culture-positive individuals from control individuals. The use of a combination of multiple ELISA targets improved the sensitivity of the assay for serological diagnosis. Elevated IgG and IgA antibody titers persisted for more than a year in the individuals with culture-confirmed pertussis.

Bordetella pertussis, the causative agent of whooping cough, is a strict human pathogen with no known animal or environmental reservoir. Pertussis vaccination has dramatically decreased the incidence of disease, but the organism continues to circulate in the human population. Severe disease is primarily seen in infants and children who have not been adequately immunized. The World Health Organization definition of pertussis is based both on clinical symptoms (a minimum of 2 weeks of paroxysmal cough) and on confirmation of infection by laboratory tests (36). The clinical symptoms of pertussis are less severe in vaccinated individuals; however, individuals with mild disease can serve as the bacterial reservoirs and act as important sources of transmission to the highly susceptible pediatric population (21, 31, 32).

The diagnosis of pertussis is challenging, and the true incidence of pertussis infection is unknown. The "gold standard" for diagnosis is isolation of *B. pertussis* from the nasopharynx. The bacterium grows very slowly and is extremely fastidious, and many laboratories cannot successfully culture the microorganism (15). PCR-based detection is more rapid and sensitive than culture-based detection (33). However, the carriage of the organism (and the presence of DNA) decreases with the duration of symptoms and with antibiotic treatment; thus, the sensitivities of both culture and PCR decrease with time (33). Serological methods can also be used for diagnosis. Enzyme-linked immunosorbent assay (ELISA) can detect *B. pertussis* infection even after the organisms are gone and is especially useful for epidemiological studies. Currently, most of the commercial ELISAs for the diagnosis of pertussis are based on antibody responses to pertussis toxin (PT) or filamentous hemagglutinin (FHA). Since both PT and FHA are included in the acellular pertussis vaccines, high levels of antibodies to these antigens could persist following vaccination, hindering serodiagnosis in vaccinated individuals. For pertussis control, immunization of adolescents is now being introduced and immunization of adults is being considered (19). Given the limitations of culture and PCR, serodiagnostic tests which can discriminate between vaccine responses and infection by *B. pertussis* will be important for monitoring the efficacies of these additional vaccine strategies to discern their impacts on disease transmission.

To address this issue we compared the performance characteristics of ELISA-based serodiagnostic tests based on five antigens not included in acellular vaccines to those of standard assays based on the vaccine antigens, PT and FHA. The nonvaccine antigens included two factors associated with the bacterial cell wall and three virulence factors. Peptidoglycan-associated lipoprotein (PAL) stabilizes the bacterial outer membrane (7). Carbonetti et al. (5) found that PAL was highly immunogenic in mice infected with a *B. pertussis* mutant lacking PT expression but was less so in mice infected with wild-type *B. pertussis*. Lipopolysaccharide (LPS) is a component of the bacterial outer membrane. The *B. pertussis* LPS has a highly branched core structure, a unique trisaccharide, and no repeating O antigen (6). Due to the lack of repeating O antigen, the LPS of *B. pertussis* has been termed lipooligosaccharide (LOS). The closely related species *Bordetella bronchiseptica* and *Bordetella parapertussis* express LOS but add a repeating O antigen, producing a conventional LPS structure (6). There are unique and shared antigenic determinants between the LOSs and LPSs of the *Bordetella* species.

The antigens derived from virulence factors of *B. pertussis* include BrkA, the C-terminal region of FHA (C-FHA), and adenylate cyclase toxin (ACT). BrkA is an adhesin and has been shown to mediate resistance to complement killing (18). FHA is a major adhesion of *B. pertussis* and is a component of

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TABLE 1. Characteristics of the culture-positive population

Visit	No. of subjects	Age (yr)		Interval between cough start and indicated visit (days [mean ± SD])
		Mean ± SD	Range	
Initial	51	11.5 ± 3.0	5–17	60 ± 30
Second	25	13.3 ± 2.5	7–18	409 ± 108
Third	3	15.7 ± 1.2	15–17	867 ± 34

most acellular pertussis vaccines. FHA is produced from a 367-kDa precursor. The first 71 amino acids comprise a signal peptide, which is removed by proteolysis. FHA is further processed by the SphB1 protease (13, 14), resulting in the 220-kDa N-terminal region that is traditionally known as FHA. Much of this form of FHA is secreted from the cell and is the source of FHA for the acellular vaccines. The C-terminal 130-kDa portion (which we term C-FHA) remains associated with the *B. pertussis* cell (13, 28) and is not included in the pertussis vaccine.

ACT is an essential virulence factor of *B. pertussis* which functions by inhibiting neutrophil defenses (35). Antibody responses to ACT have been detected in healthy individuals (1, 11), compromising its utility as a diagnostic antigen. The presence of antibodies in uninfected individuals could be due to cross-reactivity with other members of the RTX toxin family, including the *Escherichia coli* hemolysin (1, 3, 17, 22), which lacks adenylate cyclase enzymatic activity. The 400 amino acids of the N terminus of ACT (termed CatACT) comprise the catalytic domain and are unique to ACT.

Several of the nonvaccine antigens displayed good sensitivity and specificity for the serodiagnosis of pertussis and are promising candidates for the serological diagnosis of pertussis in vaccine recipients.

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from culture-positive children (79 serum samples from 51 individuals) recruited at Cincinnati Children's Hospital Medical Center from 1999 to 2002 and were characterized in a previous study (25). The characteristics of the culture-positive population are described in Table 1. Additional samples were obtained from some subjects at yearly intervals. Eighty-eight percent of the children had received the appropriate immunization series. One individual had never received pertussis vaccine. Control sera (48 serum samples from 34 donors) were collected at the University of Cincinnati from healthy adult volunteers who did not recall having a long-lasting cough in recent memory. Informed consent and assent were obtained from all subjects and their parents or guardians. This study was approved by the institutional review boards of the Cincinnati Children's Hospital Medical Center and the University of Cincinnati and was conducted according to the experimental guidelines of the U.S. Department of Health and Human Services.

Secondary antibodies. Alkaline phosphatase-conjugated goat antibody against human immunoglobulin G (IgG), IgA, and IgM were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

Antigens. We purified PT and FHA from the culture supernatant of *B. pertussis* BP338 (12, 24, 30). The LOS of BP338 and LPS of *B. parapertussis* CN8234 were purified by phenol extraction with proteinase K treatment (10, 27). Purified BrkA protein was a gift from Rachel Fernandez (26). PAL was purified from a glutathione *S*-transferase fusion protein expressed from pGEX-PAL, provided by Nicholas Carbonetti (5). The purities of all antigens were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. No contaminants were detected in any of the purified preparations.

Production of CatACT and C-FHA. A DNA fragment encoding the first 400 amino acids of the *cyaA* gene was PCR amplified from BP338 by using the oligonucleotides 5′-GCTAGCATGCAGCAATCGCATCAGGCT-3′ and 5′-AAGCTT TCACACTGCGCCCAGCGAC-3′. The open reading frame of FHA from codon

2373 to the C terminus was amplified by PCR with oligonucleotides 5′-ATATGC TAGCCAGAGCAAATTCTACGGCTCGCG-3′ and 5′-CCCAAGCTTTTTGTT GGTTTCATAGAAGACCCGGTAG-3′ with *Pfu*-turbo polymerase (Stratagene, La Jolla, CA) and 1 M GC-Melt (Clontech, Palo Alto, CA). The DNA sequences of both fragments were verified.

The fragments were cloned into the NheI and HindIII sites of pET21b (Novagen, Madison, WI), resulting in plasmid pSM021 for CatACT and plasmid pMW10 for C-FHA. Protein was expressed in Rosetta2(DE3)pLysS (Novagen), induced with isopropyl-β-D-thiogalactopyranside (2 mM), and purified from inclusion bodies.

For CatACT, lysis buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA) supplemented with lysozyme and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) was added at 4 ml/g bacteria. The bacteria were incubated for 20 min at 25°C and lysed by the addition of deoxycholic acid (4 mg/g bacteria) and sonication. The bacterial pellet was washed and dissolved in denaturing buffer (8 M urea, 50 mM Tris-HCl, pH 8.0, 0.2 mM CaCl$_2$, 0.1 mM PMSF), which was added at 8 ml/g bacteria. The urea extract was passed over a DEAE column (0.5 ml/min, 4°C), and the flowthrough was dialyzed into 25 mM sodium carbonate buffer at pH 8.0.

C-FHA was solubilized from the inclusion bodies (29) and loaded onto a HiTrap-Ni column fast-performance liquid chromatography system (Amersham Bioscience, Piscataway, NJ). The column was washed with 20 mM sodium phosphate buffer (pH 7.2) with 0.5 M NaCl and eluted with a linear gradient of imidazole (0 to 0.5 M, 20 ml).

ELISAs. ELISAs for quantitation of FHA and PT antibodies (IgG and IgA) were performed by the method of Manclark et al. (23) with Food and Drug Administration pertussis antiserum lot 3 and lot 5 as references.

For ELISAs for quantitation of protein antigens, wells of 96-well microtiter plates were coated with 100 μl of CatACT or C-FHA at a concentration of 1 μg/ml in 50 mM carbonate buffer, pH 9.6 (coating buffer), for 16 h at room temperature. Phosphate-buffered saline (PBS; pH 7.2) was used as the buffer for antigen coating for the PAL ELISA. After three washes with PBS containing 0.05% Tween 20, twofold serial dilutions of sera in incubation buffer (PBS with 10% nonfat dry milk and 0.05% Tween 20) were added at 100 μl/well. The plates were incubated at room temperature for 2 h. Secondary antibody (1:5,000) in 100 μl of incubation buffer was added, and the plate was incubated for 2 h. The wells were washed, and 200 μl of substrate (FAST-pNPP kit; Sigma, St. Louis, MO) was added. After 30 min at room temperature, 25 μl of 12% NaOH was added to the wells and the optical density at 405 nm (OD$_{405}$) was measured. Antibody values were expressed as the reciprocal serum dilution (titer) that gave an absorbance of the mean plus 2 standard deviations (SDs) of the values for the negative control wells for anti-CatACT antibodies or the reciprocal serum dilution that gave 0.5 as absorbance of the mean for anti-C-FHA and PAL antibodies.

The BrkA ELISA was performed by the procedure developed by the laboratory of R. Fernandez. In brief, the wells were coated with 100 μl of BrkA (20 μg/ml) in coating buffer with 5 mM MgCl$_2$ at 37°C for 1 h. After four washes with Dulbecco's modified PBS (DPBS), the wells were blocked with 150 μl of DPBS with 1% bovine serum albumin (BSA) at 37°C for 1 h. One hundred microliters of diluted serum (1:100) in DPBS with 1% BSA was added to the appropriate wells. After incubation at 37°C for 1 h, the wells were washed and incubated with 100 μl of secondary antibody (1:5,000) in DPBS with 1% BSA, incubated at 37°C for 1 h, and developed as described above. The antibody content was expressed as the OD$_{405}$.

For the LOS and LPS ELISAs, the wells of 96-well microtiter plates were coated with 100 μl of LOS or LPS (20 or 10 μg/ml) in coating buffer at 37°C for 2 h. The wells were washed, 300 μl of incubation buffer was added for 30 min at 37°C, and the wells were washed again. Serum diluted 25-fold in incubation buffer was added at 100 μl/well. The subsequent steps were performed as described for the CatACT ELISA, except that 1 h of incubation for sera and secondary antibodies was used. The antibody content was expressed as the OD$_{405}$.

Statistical analysis. Statistical differences between the two study groups were examined by Student's *t* test with SYSTAT11 (SYSTAT Software, Point Richmond, CA). Receiver operating characteristic (ROC) analysis was performed with Win Episcope2 (http://www.clive.ed.ac.uk/winepiscope/).

RESULTS

IgG and IgA responses to seven *B. pertussis* antigens were examined in sera from children and adolescents with culture-confirmed pertussis and a control group consisting of adult
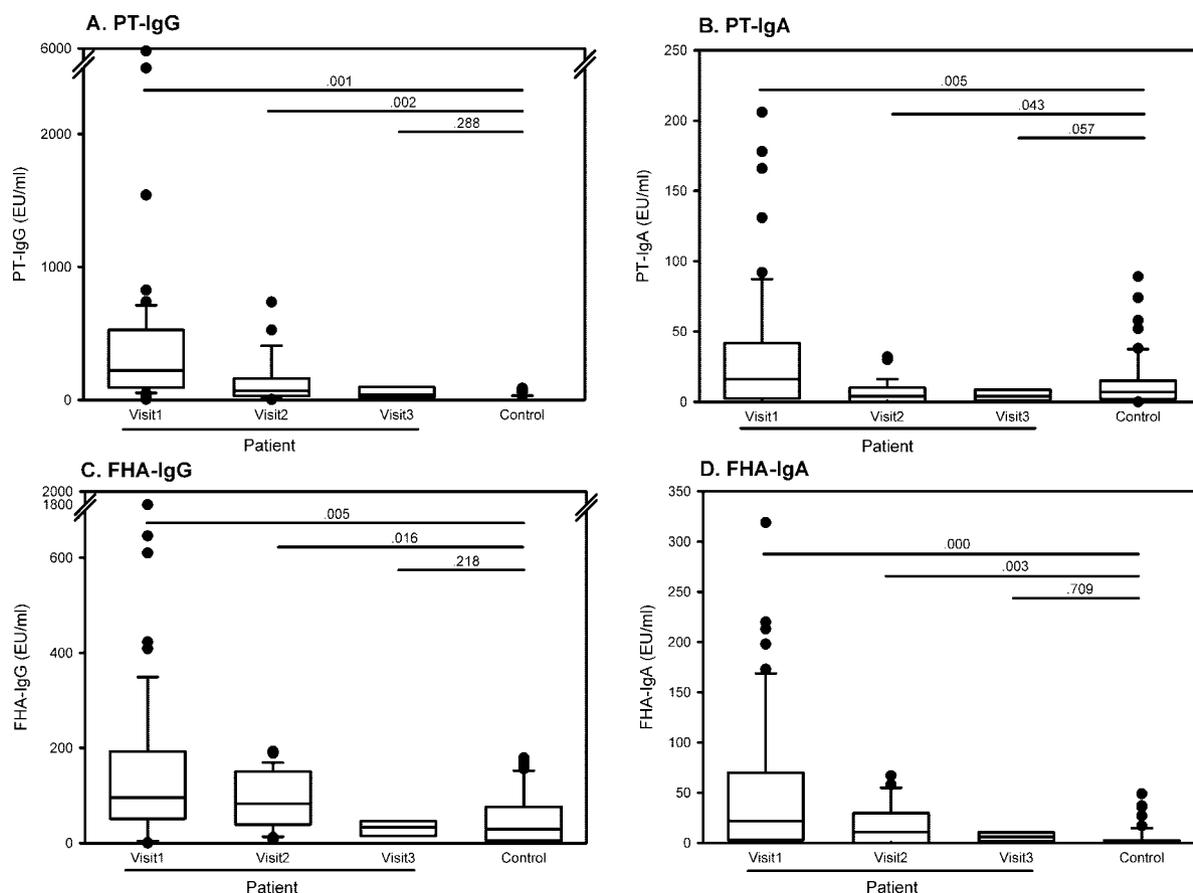
FIG. 1. Distribution of responses to vaccine antigens in the culture-positive individual group and the control group. (A) PT IgG; (B) PT IgA; (C) FHA IgG; (D) FHA IgA. Visit 1, antibody values for serum obtained at the time of diagnosis; visit 2, antibody values for serum obtained approximately 1 year after diagnosis; visit 3, antibody values for serum obtained approximately 2 years after diagnosis. Box-whisker plots represent boxes for medians with 25th and 75th percentiles and whiskers for 10th and 90th percentiles. Closed circles represent outliers. The numbers in each box chart represent the probability values obtained by Student's t test.

donors with no recollection of a recent cough disease (Table 1). In addition to the serum samples drawn at the time of diagnosis of pertussis (visit 1), we obtained serum samples approximately 1 year later (visit 2) and approximately 2 years later (visit 3) from a subset of the culture-positive individuals.

Antibody responses to vaccine antigens. PT and FHA are vaccine antigens and are also used in conventional ELISAs for the serodiagnosis of pertussis (8, 9). Consistent with previous results, the IgG and IgA antibody responses to PT were significantly higher in the culture-positive group than in the control group at both the time of diagnosis and 1 year later (Fig. 1). Mean

PT IgM levels did not differ between the culture-positive group and the control group (data not shown).

The IgG and IgA antibody responses to FHA were also significantly higher in the culture-positive group than in the control group at both the time of diagnosis and 1 year later (Fig. 1). High values of IgG antibodies to FHA were seen in the control group. This was thought to be due to proteins produced by other bacterial species that cross-react with FHA (2).

Different cutoff values have been proposed for serodiagnosis by the use of PT IgG and FHA IgG. Using the mean plus 2 SDs of the values for the control group, we calculated a value of 48

TABLE 2. Sensitivities and specificities of ELISA

Parameter	PT		FHA		CatACT		C-FHA		LOS		PAL	
	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA
Cutoff[a]	48	53	163	26	627	313	110	56	1.58	0.07	12	0
Sensitivity (%)	92.2	23.5	35.3	47.1	62.8	3.9	39.2	7.8	9.8	29.4	11.8	7.8
Specificity (%)	93.5	93.5	93.4	91.7	91.7	97.9	95.4	93.0	93.8	91.7	95.1	97.6

[a] The cutoff value for a positive result was defined as values greater than the mean plus 2 SDs of the value for the control group for all antibodies. Units for the cutoff antibody concentrations are as follows: for PT and FHA, EU/ml; for CatACT, C-FHA, and PAL, titer; for LOS, OD_{405}.
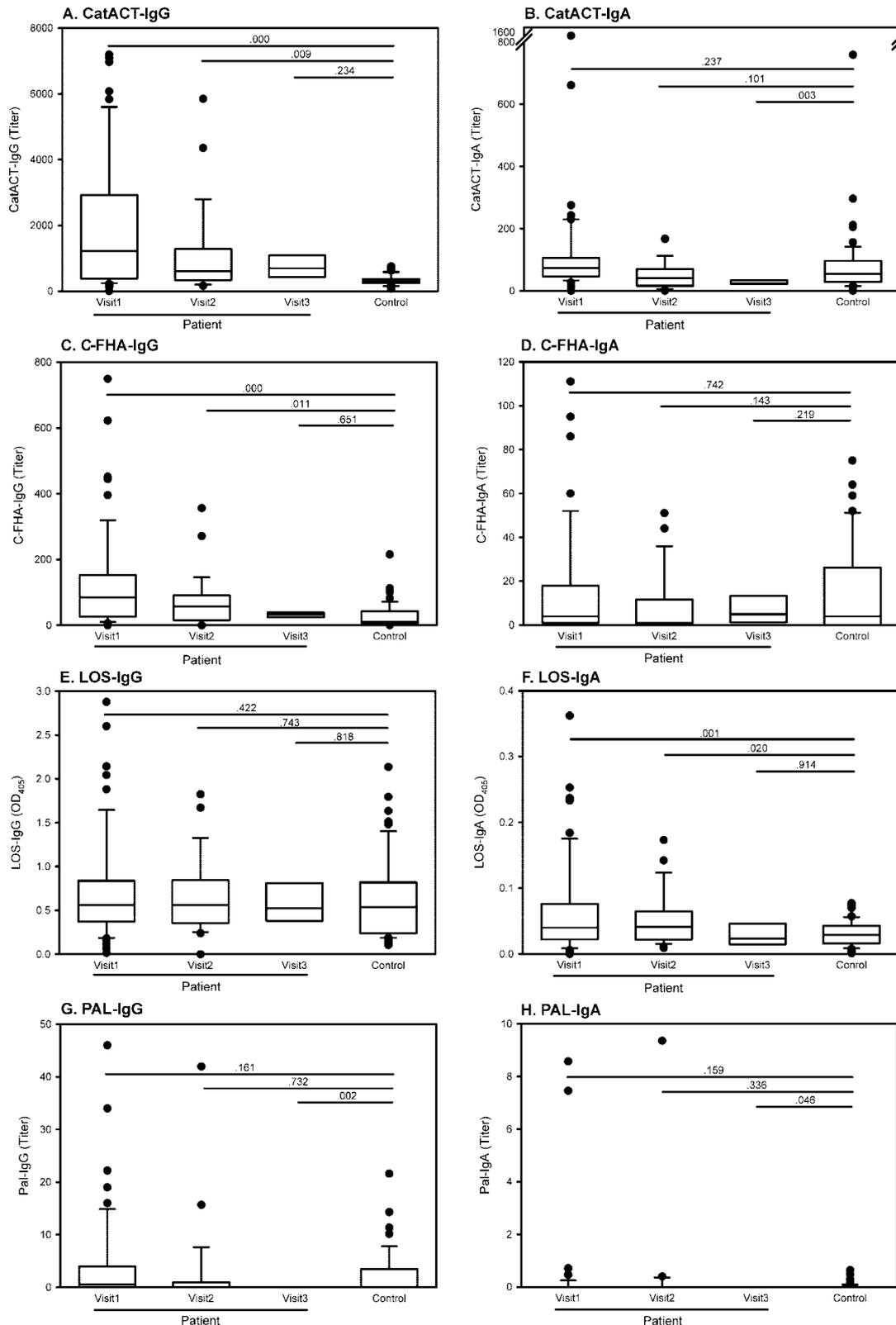
FIG. 2. Distribution of responses to nonvaccine antigens in the culture-positive individual group and the control group. (A) CatACT IgG; (B) CatACT IgA; (C) C-FHA IgG; (D) C-FHA IgA; (E) LOS IgG; (F) LOS IgA; (G) PAL IgG; (H) PAL IgA. Visit 1, antibody values for serum obtained at the time of diagnosis; visit 2, antibody values for serum obtained approximately 1 year after diagnosis; visit 3, antibody values for serum obtained approximately 2 years after diagnosis. Box-whisker plots represent boxes for medians with 25th and 75th percentiles and whiskers for 10th and 90th percentiles. Closed circles represent outliers. The numbers in each box chart represent the probability values obtained by Student's *t* test.
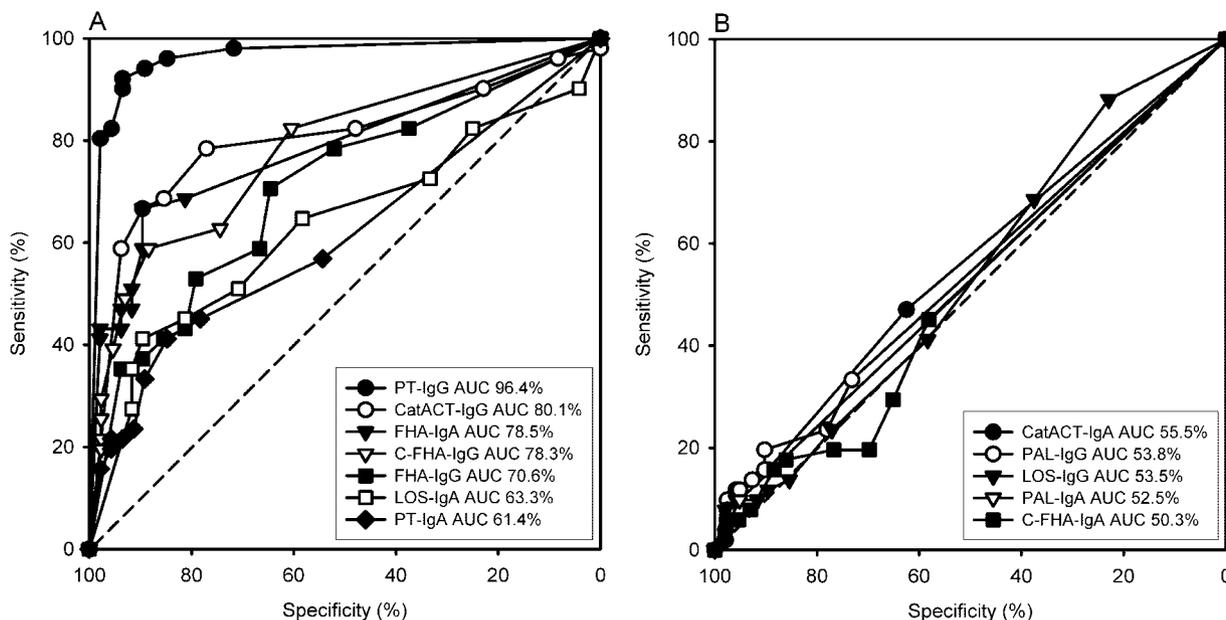
FIG. 3. ROC analysis of tests. (A) ROC curves of seven assays with predictive value (PT IgG, CatACT IgG, FHA IgA, C-FHA IgG, FHA IgG, LOS IgA, and PT IgA); (B) ROC curves of assays that lack predictive value (CatACT IgA, PAL IgG, LOS IgG, PAL IgA, and C-FHA IgA).

ELISA units (EU)/ml to be the cutoff value for seropositive responses to PT, giving a specificity of approximately 95% (Table 2). This value is more than double the traditional definition of 20 EU/ml (4). Under the traditional cutoff definition, approximately 13% of the control group would be considered seropositive, reducing the specificity of the test to 87%. In contrast, Baughman et al. (4) proposed a cutoff value of 94, and this value would reduce the sensitivity of the PT IgG assay to 73%. The sensitivity of the FHA IgG test was only 35.3% by the use of our calculated cutoff value of 163 EU/ml (Table 2). This value also falls between the conventional value, 20 EU/ml, and a much higher value, 229 EU/ml, suggested by Baughman et al. (4). The conventional cutoff value reduced the specificity of FHA IgG to 39.6%, while the higher value reduced the sensitivity to 15.7%.

Antibody responses to nonvaccine antigens. The responses to five nonvaccine antigens were also characterized (Fig. 2). The mean IgG responses to CatACT (Fig. 2A) and C-FHA (Fig. 2C) of the culture-positive individuals were higher than those of the control group at the time of diagnosis and 1 year later. However, the IgA responses to CatACT (Fig. 2B) and C-FHA (Fig. 2D) were not different between the two groups. The sensitivity and specificity of CatACT IgG as a diagnostic tool were 62.8% and 91.7%, respectively (Table 2), while the sensitivity and specificity of C-FHA IgG as a diagnostic tool were 39.2% and 95.4%, respectively (Table 2). Mean CatACT IgM levels did not differ between the culture-positive group and the control group (data not shown).

The IgG response to LOS was not different between the culture-positive group and the control group (Fig. 2E), and interestingly, the LOS IgG titers did not decrease with time. In contrast, the LOS IgA titers did differ between the two groups. The sensitivity of LOS IgA as a diagnostic tool was relatively low, 29.4% (Table 2).

Two proteins, BrkA and PAL, did not appear to be very immunogenic. The titers of IgG antibody against BrkA were measured in only six culture-positive individuals and six control individuals (data not shown). Most of the individuals had no detectable response to BrkA, and there was no mean difference between the culture-positive individuals and the control individuals ($P = 0.226$). Similarly, most of the subjects did not have antibodies to PAL, and the responses for the culture-positive individuals did not differ from the responses of the control group (Fig. 2G and H).

Comparison of test results. All possible combinations of sensitivity and specificity that can be achieved by changing the test's cutoff value can be summarized by using the area under the curve (AUC) of a ROC curve (20). An assay with an AUC of 50% represents an assay that has no discriminating ability, while an AUC of 100% means that the assay can provide perfect discrimination. The test for PT IgG had the largest AUC (Fig. 3) and was the best diagnostic test identified in this study. CatACT IgG, FHA IgA, C-FHA IgG, FHA IgG, LOS IgA, and PT IgA were less sensitive but were still useful for the diagnosis of pertussis (Fig. 3A). In contrast, for CatACT IgA, C-FHA IgA, LOS IgG, PAL IgG, and PAL IgA (Fig. 3B), the AUCs were about 50% and their 95% lower confidence limits were less than 50%, suggesting that tests for these antibodies have little ability to discriminate infected individuals from healthy controls as stand-alone diagnostic tests.

The definition of a positive diagnostic test result as an ELISA value greater than the mean plus 2 SDs of the values for the control group resulted in greater than 90% specificity for all assays (Table 2). The PT IgG-based assay had the highest sensitivity (92.2%), followed by the CatACT IgG-based assay, with 62.8% sensitivity. The CatACT IgA, C-FHA IgA, LOS IgG, and PAL IgA and IgG antibodies had very low sensitivities (nearly 10% or less).

TABLE 3. Diagnosis based on the results of multiple tests

Test and antibody	Sensitivity (%)	Specificity (%)
Conventional ELISAs		
PT IgG	92.2	93.5
FHA IgG	35.3	93.4
PT IgG or FHA IgG	92.2	93.5
Novel ELISAs		
CatACT IgG	62.8	91.7
PT IgG or CatACT IgG	96.1	95.8
PT IgG, CatACT IgG, or FHA IgA	98.0	79.2
CatACT IgG or C-FHA IgG	72.5	87.5
CatACT IgG, C-FHA IgG, or LOS IgA	80.4	83.3

Diagnosis based on multiple tests. Combinations of different ELISAs are often used to improve sensitivity. When the results of all 14 tests are considered, the mean number of positive results for the culture-positive individuals was 3.7 (median and mode = 4), with a range from 0 to 9. Only one person was negative by all 14 tests. The combination of PT IgG and FHA IgG detection is widely used; however, all of the FHA IgG-positive patients were also positive for PT IgG, and this combination did not improve the test performance (Table 3). A combination of three assays with nonvaccine antigens (CatACT IgG, C-FHA IgG, and LOS IgA) detected 41 of 51 (80%) culture-positive individuals, and the specificity was 83%.

When the results of all 14 tests are considered for the control group, the mean number of positive results was 0.7 (median and mode = 0), with a range from 0 to 4. Ten serum samples in the control group were positive by two or more tests.

Duration of antibody responses. We also examined the status of individuals a year after diagnosis. By the PT IgG assay, 92.0% of the culture-positive individuals (23 of 25) were seropositive at the time of diagnosis and 64.0% were positive (16 of 25) after 1 year. For the CatACT IgG assay, 68.0% (17 of 25) of the culture-positive individuals were seropositive at the time

of diagnosis and 44.0% (11 of 25) were positive after 1 year. Similarly, 48.0% (12 of 15) of the culture-positive individuals were seropositive by the FHA IgA assay at the time of diagnosis and 28.0% were seropositive (7 of 25) after 1 year.

Immune response to *B. parapertussis* LPS. The antibody responses to the LPS of *B. parapertussis* were also measured. Both groups had high levels of LPS IgG, and the distribution of the two groups overlapped (Fig. 4). In the test for IgA LPS, eight individuals in the culture-positive groups and one control individual were positive for the LPS of *B. parapertussis* test under the cutoff definition of the mean plus 2 SDs of the values for the controls. No correlation was detected between IgA against LOS of *B. pertussis* and IgA against LPS of *B. parapertussis*.

DISCUSSION

The onset of pertussis is very slow, and individuals often harbor the microorganisms for weeks before they display clinical symptoms of pertussis. This is long enough for antibody responses to develop, making the serodiagnosis of pertussis especially useful. Until recently, only children received the pertussis vaccine. Immunization of adolescents is now being introduced, and immunization of adults is being considered (19). While PT and FHA have been shown to be useful for serodiagnosis for individuals who have not been vaccinated for pertussis, PT and FHA are contained in most acellular pertussis vaccine formulations and are not likely to be useful for the diagnosis of disease in recently vaccinated individuals. In addition to PT and FHA, we examined the sensitivities and specificities of IgA and IgG serum antibody responses to five *B. pertussis* antigens that are not currently used in acellular pertussis vaccines. Seven tests, those for PT IgG, CatACT IgG, FHA IgA, C-FHA IgG, FHA IgG, LOS IgA, and PT IgA, appeared to be promising for the serodiagnosis of pertussis as stand-alone diagnostic tests. By using our cutoff value of 48 EU/ml, the PT IgG titer was by far the most sensitive and
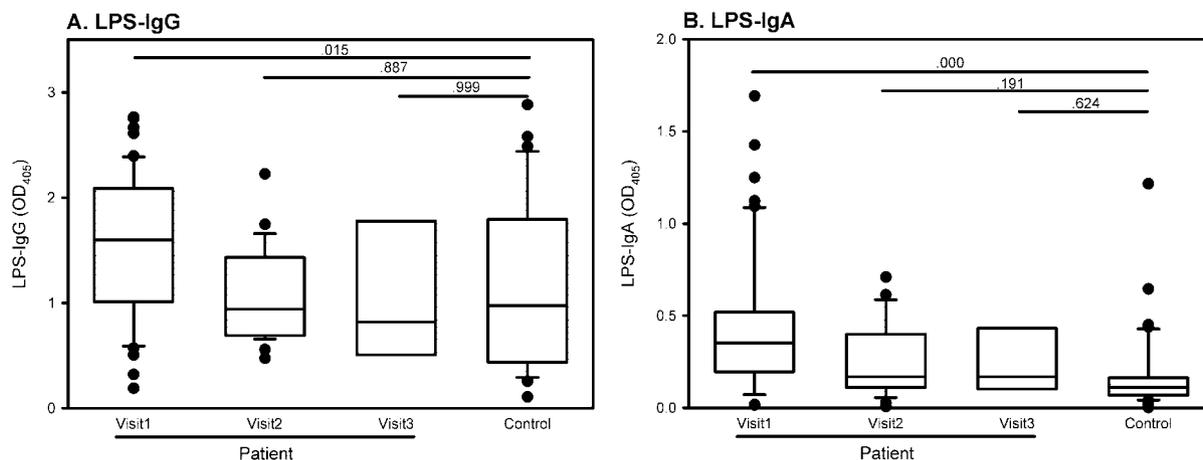

FIG. 4. Distribution of responses to LPS of *B. parapertussis* in the culture-positive individual group and the control group. (A) LPS IgG; (B) LPS IgA. Visit 1, antibody values for serum obtained at the time of diagnosis; visit 2, antibody values for serum obtained approximately 1 year after diagnosis; visit 3, antibody values for serum obtained approximately 2 years after diagnosis. Box-whisker plots represent boxes for medians with 25th and 75th percentiles and whiskers for 10th and 90th percentiles. Closed circles represent outliers. The numbers in each box chart represent the probability values obtained by Student's *t* test.

specific indicator of infection. PT has the added advantage of being uniquely expressed by *B. pertussis* and can discriminate between infection by *B. pertussis* and the antigenically related but less virulent species *B. parapertussis*. The assay for CatACT IgG was the second most sensitive test. Cherry et al. suggested that the failure of antibody responses to intact ACT to correlate with infection was due to the presence of antigenic domains shared with other bacterial toxins (11). Using the unique portion of ACT, CatACT, we found the IgG antibody response to CatACT could discriminate between culture-positive individuals and control individuals. CatACT IgG is the best single candidate for use in a serological diagnostic test that is not affected by vaccination status, and use of the CatACT IgG test in combination with other tests based on nonvaccine antigens improved the sensitivity.

B. parapertussis can also cause pertussis-like illness. Current pertussis vaccines have little efficacy against *B. parapertussis* infection (34). Nine individuals (eight patients and one control) were positive by the *B. parapertussis* LPS IgA ELISA. This was likely due to past infection with *B. parapertussis* and not cross-reactivity to *B. pertussis* LOS, since the individuals who were positive for LOS IgA of *B. pertussis* were frequently not positive for LPS IgA of *B. parapertussis*, and vice versa. Correlation analysis revealed there was no significant correlation between *B. parapertussis* LPS IgA and *B. pertussis* LOS IgA ($P = 0.154$).

Our data suggest that serum antibodies to pertussis antigens may persist for a year or more following infection. At present it is not possible to discriminate recent infection from infection that may have occurred years ago. IgA antibodies have a shorter serum half-life (6 days for IgA versus 23 days for IgG1) and are thought to be a more specific indicator of recent infection. Our data suggest this may not be the case, since elevated IgA responses to FHA were detected 1 year later. Interestingly, 9 of 34 or 26% of the adults in the control group displayed serological evidence of infection (which we defined as two or more test responses greater than the mean + 2 SDs), even though they did not report a recent cough illness. This result supports the findings of studies that suggest that mild cases of pertussis may be common in the adult population (21, 31, 32).

The population used in this study was one in transition with regard to pertussis immunization. The samples from the culture-confirmed cases were collected from individuals who were 5 to 17 years old from 1999 to 2002. The older subjects likely received exclusively whole-cell vaccine, a few of the younger subjects could have received exclusively acellular pertussis vaccine, and many of the subjects would have received a mixed series. It would be important to repeat this study with samples from a population immunized with acellular pertussis vaccine by using age-matched controls.

In summary, we have identified several nonvaccine antigens that could be used for the serodiagnosis of pertussis. Booster vaccination of adolescents and adults is being considered. Diagnostic tests for pertussis based on nonvaccine antigens would be useful for monitoring of the epidemiology of pertussis and the effectiveness of immunization in preventing pertussis.

ACKNOWLEDGMENTS

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