JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1984, p. 891–893
0095-1137/84/110891-03$02.00/0

Vol. 20, No. 5

Importance of Culture in Laboratory Diagnosis of *Bordetella pertussis* Infections

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Received 4 June 1984/Accepted 6 August 1984

Nasopharyngeal secretions from 223 patients were examined for the presence of *Bordetella pertussis* and *B. parapertussis* by culturing on Regan-Lowe agar (J. Regan and F. Lowe, J. Clin. Microbiol. 6:303–309, 1977) and by direct fluorescent-antibody testing. *B. pertussis* was found in 38 patients; *B. parapertussis* was recovered from 2. Culturing was more sensitive (38 of 38 patients) than direct fluorescent-antibody testing (26 of 38 patients) for the detection of *B. pertussis*. Overgrowth by other organisms (7 of 223 patients) was uncommon. The patients with *B. pertussis* infections were generally less than 1 year old, had received no or one immunization, and had coughing spells but infrequently had whooping cough. Accurate diagnosis of *B. pertussis* infections should include culturing.

With the successful establishment of a nationwide immunization program in the United States against diphtheria, pertussis, and tetanus, the prevalence of these diseases fell dramatically. They have not been eliminated, however. Of the three diseases, pertussis is by far the most frequent, with ca. 1,500 to 3,000 cases reported to the Centers for Disease Control, Atlanta, Ga., annually (5). *Bordetella pertussis* infection is diagnosed most frequently and is most severe in infants under 6 months old (9, 11). *B. pertussis* infection is highly contagious and is rapidly spread to susceptible individuals. A decline in vaccine acceptance because of concern among parents about the safety of whole-cell pertussis vaccines may result in large outbreaks of pertussis in the United States, as was observed in the United Kingdom and Japan during the mid-1970s (11, 13).

Until vaccine efficacy and acceptance are universal, the control of *B. pertussis* infection depends upon the early identification and treatment of patients with pertussis and their contacts. We present data which indicate that optimal laboratory techniques to confirm the diagnosis of *B. pertussis* infection must include culturing as well as direct fluorescent-antibody (DFA) testing.

MATERIALS AND METHODS

Patient population. Children presenting to St. Christopher's Hospital for Children between May 1982 and August 1983 with symptoms suggestive of pertussis were evaluated by means of nasopharyngeal culturing and smears. The primary symptom was a cough, sometimes accompanied by cyanosis and choking or followed by whooping and vomiting; some patients had only mild upper respiratory tract involvement, with rhinorrhea and congestion. Both culturing and DFA testing were performed on nasopharyngeal secretions from all patients. The medical records of patients with positive results by either method or both methods for either *B. pertussis* or *B. parapertussis* were reviewed. Families were contacted by telephone several months after the child's illness to determine the duration of illness.

Culturing and DFA testing. Nasopharyngeal specimens were obtained with calcium alginate nasopharyngeal swabs. Three to six slides were prepared at the bedside for DFA testing. Specimens for culturing were transported to the laboratory in 1% Casamino Acids. Upon arrival in the laboratory, each specimen was inoculated immediately onto charcoal agar (Oxoid Ltd., Columbia, Md.) supplemented with 10% sheep blood and 40 μg of cephalexin per ml, as described by Regan and Lowe (12). The cultures on Regan-Lowe medium were incubated at 35°C in 5 to 10% CO_2. The cultures were examined daily for 7 days for the presence of small, round, glistening colonies, which usually appeared after 2 to 4 days of incubation. These organisms were confirmed to be *B. pertussis* or *B. parapertussis* by the DFA techniques described below.

Each of three slides per patient were stained, usually within 24 h of arrival in the laboratory, with fluorescein-labeled chicken anti-*B. pertussis* globulin and anti-*B. parapertussis* globulin and goat antirabbit globulin, in accordance with the manufacturer's (see below) instructions. A DFA test was considered positive for *B. pertussis* or *B. parapertussis* when short rods with a rim of bright-green fluorescence were visualized with the respective antiglobulins and no fluorescence was observed with the goat antirabbit globulin. The fluorescein-labeled antibodies against *B. pertussis* and *B. parapertussis* were purchased from Difco Laboratories, Detroit, Mich., whereas the goat antirabbit globulin, which was used as a negative control for detecting nonspecific fluorescence, was obtained from BBL Microbiology Systems, Cockeysville, Md.

RESULTS

B. pertussis was detected in 38 of the 223 patients studied, whereas *B. parapertussis* was detected in only 2 of the 223 (Table 1). Culturing on Regan-Lowe medium was more efficient than DFA testing in detecting both *B. pertussis* and *B. parapertussis*. Twelve patients were culture positive and DFA negative for *B. pertussis*, and one patient was culture positive and DFA negative for *B. parapertussis*. No patient was DFA positive and culture negative for either *B. pertussis* or *B. parapertussis*. There were no instances in which DFA testing was positive for both *B. pertussis* and *B. parapertussis*. Upon initial testing, 1 of the 38 isolates of *B. pertussis* reacted with both specific conjugates. However, a repeat testing of the isolate resulted in it reacting with *B. pertussis*

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TABLE 1. Comparison of DFA testing with culturing for the detection of *B. pertussis* and *B. parapertussis*

Result[a]	No. of patients infected with:	
	B. pertussis	*B. parapertussis*
DFA +, culture −	0	0
DFA −, culture +	12	1
DFA +, culture +	26	1
DFA −, culture −	178	214
DFA −, culture overgrown	7	7

[a] DFA testing was done with conjugated antiserum against *B. pertussis* and *B. parapertussis*. Culturing was done on Regan-Lowe medium containing 40 μg of cephalexin per ml.

conjugate only; this isolate was determined to be *B. pertussis*. Overgrowth of the Regan-Lowe medium by oropharyngeal flora was uncommon, occurring in only 7 of the 223 cases. When overgrowth did occur, it was caused by heavy growth of pseudomonads, yeasts, or molds.

Medical records were available for 37 of the 38 patients positive for *B. pertussis* and for both of the patients with *B. parapertussis* infections. The age of the patients with *B. pertussis* infections ranged from 3 weeks to 13 years, with 31 of 37 (84%) being less than 12 months old. Of those 31, 12 (32%) were 0 to 2 months old, 10 (27%) were 2 to 6 months old, and 9 (24%) were 6 to 12 months old. The two patients with *B. parapertussis* infections were 1 and 2 months old. The diphtheria-pertussis-tetanus immunization status of patients with *B. pertussis* infections was as follows: 12 had received no immunization, 14 had received one, 6 had received two, 2 had received three, 1 had received five, and 2 were unknown. Neither patient with a *B. parapertussis* infection had received diphtheria-pertussis-tetanus immunization.

Leukocyte counts and differentials were determined for 30 of the 37 patients. Leukocyte counts exceeded 10,000 cells per mm^3 in 25 of the 30 patients and 20,000 cells per mm^3 in 13 of the 30 patients. Lymphocytosis of >50% was observed in all patients; 14 of the 30 patients had >70% lymphocytes. Atypical lymphocytosis was not observed. Chest X-rays were abnormal for 28 of the 30 *B. pertussis*-positive individuals. Overaeration and peribronchial thickening were the most frequent findings. Both *B. parapertussis* patients had abnormal chest X-rays. The leukocyte count and differential were normal for the *B. parapertussis*-positive patient for whom these tests were performed.

The clinical features of the patients with *B. pertussis* infections are shown in Table 2. Almost all of the patients with *B. pertussis* infections had a cough, but only 16% had the characteristic whoop associated with this disease. Choking and cyanosis were more common, with 24 and 27% of the patients, respectively, having these symptoms. The duration of the cough was determined for 15 patients. Of the 15, 12 had a cough for a minimum of 2 weeks after diagnosis, and 1 had a cough for 32 weeks after diagnosis. The median length of the cough was 4 weeks.

When age, immunization status, laboratory findings, and clinical features were compared for the DFA-positive and DFA-negative pertussis patients, there were no statistically significant differences.

Twenty-four of the *B. pertussis* patients were hospitalized for observation and therapy. The length of hospitalization ranged from 2 to 21 days, with a median length of stay of 4 days. Complications associated with *B. pertussis* included six cases of otitis media, three cases of pneumonia, and one case of apnea requiring mechanical respiratory assistance. There were no deaths. One of the two patients with a *B. parapertussis* infection was hospitalized for 2 days. Both had a cough, but neither had a whoop, choking, or cyanosis. The duration of the cough was 5 and 7 days for the two patients.

DISCUSSION

Pertussis was an important cause of infant morbidity and mortality in the United States until the development and widespread administration of whole-cell *B. pertussis* vaccines in the mid-1950s. Recently, there has been concern both in the United States (7, 14) and in other developed countries (8, 15) about the safety of the vaccine. The general public has been especially wary of immunization because of reports of postvaccine encephalopathy and sudden death (15). Because of these concerns, vaccine acceptance has dropped in both Great Britain and Japan, with resulting large epidemic outbreaks of pertussis and attendant large increases in morbidity and mortality associated with this disease. There has been increasing worry among health care officials in the United States that there may be a large increase in the number of cases of pertussis here as a result of declining pertussis vaccine acceptance (6).

A major problem in studying the epidemiology of pertussis is making an accurate laboratory diagnosis. Several viral syndromes may be confused with both *B. pertussis* and *B. parapertussis* infections. Prophylaxis with erythromycin can prevent the spread of *B. pertussis* and *B. parapertussis* infections, whereas prophylactic treatment is not usually

TABLE 2. Clinical features of 37 patients with *B. pertussis* infections

Clinical feature	% of patients with feature
Duration (days) of symptoms before diagnosis	
0 to 5	25
6 to 10	44
11 to 60	31
Cough	95
Symptoms associated with cough	
Whoop	16
Choking	24
Cyanosis	27
Vomiting	11
Duration (weeks) of cough after diagnosis (n = 15)	
<2	20
2 to 4	47
>4	33
Patients requiring hospitalization	65
Duration (days) of hospitalization (n = 24)	
<3	46
4 to 7	29
>7	21
Unknown	4
Complications	
Otitis media	16
Pneumonia	8
Apnea	3
Deaths	0

used to contain the spread of viral infections. Because of this, techniques for the laboratory diagnosis of *B. pertussis* and *B. parapertussis* infections should be sensitive, specific, and, if possible, rapid, so that prophylaxis can be used when appropriate.

Three approaches can be used to diagnosis pertussis: culturing, DFA testing, and serology. In this study, culturing on Regan-Lowe agar and DFA testing were compared, and culturing was found to be more sensitive than DFA testing for the detection of *B. pertussis* and *B. parapertussis*. This finding is in agreement with those of Regan and Lowe (12), except that we found DFA testing to be more sensitive for the detection of the two organisms than Regan and Lowe did. In earlier studies, when culturing with Bordet-Gengou (BG) agar was compared with DFA testing, DFA testing was found to be more sensitive than culturing for the detection of *B. pertussis* (2, 4). However, it was not possible to determine in these earlier studies the number of false-positive DFA results obtained. Both false-positive and false-negative DFA results can be obtained because of the variation in the interpretation of results by different investigators, as has been shown by Broome et al. (3). In our study, there were no culture-negative and DFA-positive patients. This was probably because of the facts that >90% of the DFA results were read by the same person, the patients were usually tested early in the course of disease (Table 2), and the patients had not received prior appropriate antimicrobial therapy. It has been documented that patients who have been treated with erythromycin can remain DFA positive after becoming culture negative (1). We found that DFA testing was useful when it was positive because it was very specific, that false-positives were not reported, and that results were available on the same day, whereas culturing was rarely positive within 48 h of plating. Because DFA testing was not as sensitive as culturing, negative results should be interpreted with caution.

BG agar supplemented with penicillin or methicillin has been recommended as the medium of choice for the isolation of *B. pertussis* and *B. parapertussis* (10). This medium is very inconvenient to use in most clinical laboratories because, for optimal recovery of *B. pertussis*, it must be made on the day of culturing. When commercially prepared BG agar was compared with Regan-Lowe agar in 25 patients with *B. pertussis* infections, the organism was recovered from 4 of 25 patients when BG agar was used and from all 25 patients when Regan-Lowe agar was used (F. Chan, E. Rossier, A. M. R. MacKenzie, and A. Comos, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C260, p. 355). Regan-Lowe agar has the added advantages over BG agar of an 8-week shelf life and less overgrowth by other organisms (7 of 223 cases). Because of its sensitivity, selective characteristics, and extended shelf life, Regan-Lowe agar would appear to be a useful alternative to freshly prepared BG agar in most clinical laboratories. Comparative studies are needed to determine if Regan-Lowe agar is superior to freshly prepared BG agar.

The clinical features of our patients were consistent with those seen in one other recent outbreak of pertussis (9). The children in that study were usually less than 1 year old and had received no or one pertussis vaccine dose, and only a few had the characteristic whoop which is the hallmark of this infection. Over half the children required hospitalization (hospitalization was required mainly in very young children with this infection). This was not surprising, as most of the morbidity and mortality associated with *B. pertussis* occurs in this age group (8, 11). The disease in patients infected with *B. parapertussis* appeared to be less severe, but the group was too small for a meaningful comparison to be made with the group infected with *B. pertussis*.

B. pertussis continues to be an organism of public health concern in the United States. For assessment of the role of this organism in respiratory diseases of young children, techniques for the detection of *B. pertussis* and *B. parapertussis* should be sensitive and specific. Any attempts to make this diagnosis should include culturing. Data which showed that culturing on Regan-Lowe agar was more sensitive than DFA testing and that this medium should be considered as an acceptable alternative to BG agar for the isolation of *B. pertussis* and *B. parapertussis* have been presented.

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