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Diagnosis of Whooping Cough—A Comparison of Culture, Immunofluorescence and Serology with ELISA

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Three methods for diagnosis of whooping cough—culture, immunofluorescence (IF) technique on nasopharyngeal secretion and serology with ELISA—were compared. 52 patients with symptoms of upper respiratory tract infection, which could not exclude pertussis as a differential diagnosis, were investigated. Pertussis infection was confirmed in 30 patients. Of these 16 (53 %) were found by culture, 19 (63 %) by IF and 28 (93 %) by serology. It is concluded that IF analysis of nasopharyngeal secretion is a valuable tool for rapid diagnosis of whooping cough with a sensitivity similar to that of culture. Serodiagnosis with ELISA added a significant number of positive patients in which culture and IF were negative.

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INTRODUCTION

Reliable and rapid confirmation of whooping cough is essential for epidemiological reasons and for successful outcome of antibiotic treatment. The culture of Bordetella pertussis is slow and because of the method's low diagnostic sensitivity, a negative result still does not exclude the diagnosis.

In the acute situation, immunofluorescence (IF) technique for visualization of bacteria in nasopharyngeal secretion may be of great value. Several investigators have compared these two methods and the sensitivity was approximately equal when routine handling of the specimen was employed (3, 4, 8). Immediate processing, avoiding transport medium, could slightly raise the sensitivity of culture (3). However, the IF technique has been questioned because of the lower specificity (3), yielding a proportion of false positives estimated to about 10% (4).

The most sensitive method for diagnosing pertussis is to measure the antibody response to the fimbrial hæmagglutinins of B. pertussis with enzyme-linked immunosorbent assay (ELISA) (7). This technique allows separate evaluation of IgG, IgM and IgA antibody response, and a diagnostic sensitivity of approximately 95% is achieved provided acute and convalescent sera are compared (6, 7). Analysis of acute serum alone yields a sensitivity slightly better than that of culture, but the result cannot generally be presented any faster (6).

The purpose of this prospective study was to compare the outcome of the 3 diagnostic methods—culture, IF on nasopharyngeal secretion and serology with ELISA—in patients presenting signs and symptoms possibly attributable to whooping cough.

MATERIALS AND METHODS

The investigation was carried out between November 1981 and April 1982 at the Departments of Infectious Diseases and Pediatrics at the Central Hospital, Eskilstuna. All patients presenting a clinical picture compatible with pertussis were enrolled in the study. In a few cases symptomfree

contacts were included. The total number of patients was 52. 33 of these were born in 1979 or later. These children were not vaccinated against pertussis since the national vaccination programme was abandoned after 1978. 14 patients were born between 1978 and 1972 and 5 patients were adults.

At the initial consultation a nasopharyngeal swab for culture and IF analysis was obtained. Also a capillary blood specimen was collected. After separation, the serum was stored at −20°C awaiting the convalescent serum which was obtained after another 6–8 weeks. Culture and IF analysis were performed at the bacteriological laboratory of the Central Hospital, Eskilstuna, usually the same day and always within 24 h. The ELISA was performed at the National Bacteriological Laboratory, Stockholm.

Culture was achieved by inoculation of swabs onto Bordet-Gengou agar plates (Oxoid), supplemented with 1% glycerol and 17% human blood. Inoculations were done in duplicate onto one agar plate also containing cloxacillin (0.85 mg/l), the other plate being without supplementation with antibiotics. The plates were inspected daily and incubated for a total of 7 days. Isolates were verified by morphology, agglutination and biochemical characteristics (9).

IF analysis was performed after the swab had been used for inoculation of agar plates. The swab was placed in a small test tube containing 0.5 ml of sterile water and a few glass beads. The tube was shaken on a mixer for 1 min and 2 drops of approximately 0.1 ml each were placed on a glass slide adopted for IF technique, containing 8 clear wells with black surrounding (AB Novakemi, Stockholm). The drops were allowed to dry and after fixation in absolute ethanol, antibody staining ensued according to the directions of the manufacturer of the FITC-conjugates (Difco Laboratories, Detroit, USA). Each specimen was stained with conjugate against B. pertussis and B. parapertussis. Simultaneously, positive and negative controls were run.

The ELISA has been previously described in detail (2). In short, the fimbrial hæmagglutinins used as antigen in the assay were obtained by extraction of the bacteria with 1 M sodium acetate and purification by gel chromatography on a Sephacryl® S-300 column (Pharmacia Fine Chemicals, Uppsala, Sweden). Specific alkaline phosphatase-conjugated antisera to human IgG, IgM and IgA were used (Orion Diagnostica, Helsinki, Finland). A significant rise of titer was defined as a 2-fold increase in titer as compared to the previous sample. High titers of IgG and positive titers of IgM and IgA antibody were defined as >2 SD of the mean titers found in a healthy population of the same age group. The assay is specific for antibodies against the fimbrial hæmagglutinins of B. pertussis and B. parapertussis (7).

RESULTS

Of the 52 patients, 33 were positive in one or more tests. In 3 patients with positive IF only, the diagnosis was not considered fully confirmed. Of the 30 patients with established pertussis infection, 16 were found by culture (53%), 19 by IF (63%) and 28 were found seropositive in the ELISA (93%). Of the 28 seropositive patients, 26 had significant rise of titer in one or more of the Ig-classes or high IgG titer with positive IgM. Two patients had

Table I. *Outcome of diagnostic methods for pertussis*

No. of patients		Outcome of test: Culture	IF	ELISA
13		+	+	+
2		+	+	−
1		+	−	+
4		−	+[a]	+
3		−	+[b]	−
10		−	−	+
19		−	−	−
Total 52	Total no. of pos.	16	19	28

[a] One patient was IF positive for B. parapertussis.
[b] Excluded from the group of patients with fully confirmed diagnosis.

high IgG titer only without significant rise. These 2 were also negative in culture and IF. 19 patients were negative in all 3 tests. The outcome of the tests is detailed in Table I.

The proportions of true positives in each pair of groups were compared with McNemar's test (1). Accordingly, the difference between culture and IF was not significant whereas culture vs. ELISA and IF vs. ELISA rendered a p value <0.05.

DISCUSSION

From the clinical records of this group of patients it is obvious that the course of pertussis infection is often atypical and that symptoms may be indistinguishable from upper respiratory tract infection of other etiology. 23 of the patients would have been considered positive if only the standard diagnostic methods, culture and IF, had been used. With the ELISA another 10 patients with whooping cough were disclosed. Furthermore the ELISA was eventually helpful in indicating patients who needed to be considered at risk for a falsely positive IF result.

In 2 patients culture and IF were positive whereas the antibody response was negative. One of these was the wife in a family where the husband and daughter had clinical symptoms and the diagnosis could be confirmed with laboratory methods. This woman had no symptoms of disease and remained healthy without treatment with antibiotics. The other patient was a 10-yr-old boy with vague symptoms who was treated early with erythromycin on basis of a positive epidemiology. This shows that a person may be colonized with B. pertussis without developing the disease or raising a systemic immune response. Furthermore it is suggested that some individuals, treated early with antibiotics, are not adequately stimulated by bacterial antigens to mount a systemic immune response. This phenomenon was also noted in a previous study (6). It is unknown whether these individuals are still susceptible for renewed infection.

In 26 of the 28 seropositive patients the antibody response was 100% indicative of pertussis infection, i.e. significant rise of titer or high IgG titer in combination with positive IgM. In 2 patients constantly high IgG titers were found. This antibody response pattern is at least 90% indicative of recent disease (7). Both patients had classical symptoms of whooping cough, and one had also positive epidemiology (family contact to culture verified patient). Therefore we felt it reasonably safe to classify these 2 patients as true seropositives.

One patient with significant rise of titer was IF positive for B. parapertussis. Because of the structural similarity of the fimbrial hæmagglutinins of B. pertussis and B. parapertussis the ELISA does not discriminate between the two (7). We have included this patient since the disease caused by B. parapertussis is often indistinguishable from true pertussis and treatment is the same. In fact, it was recently suggested that the differences between the two species are merely academic (5).

In 3 patients IF was the only positive diagnostic method, and in these we did not consider the diagnosis fully established, being aware of the risk for falsely positive IF results (3, 4). However, nor could they all be unequivocally dismissed as false positives. No one of these patients had a positive epidemiology for pertussis. A 1-yr-old boy ran a clinical course which was not suggestive of whooping cough and he also had a history of contact with persons with upper respiratory tract infection of virus etiology. One young woman had a history of pertussis-like coughing for 5 weeks at the initial consultation. She was not treated with antibiotics. The third patient was an 18-month-old boy with a short history of typical whooping cough symptoms and he was treated with erythromycin. Thus, we have considered the first of these patients as probably falsely positive in IF. In the other 2 the clinical record, although being a weak piece of evidence, could not exclude the

diagnosis. Therefore, in the group of 22 IF positive patients the risk for a falsely positive result would be at least 5% (1/22) and at most 14% (3/22), yielding a specificity of the IF technique in the range between 86 and 95%.

In conclusion, IF constitutes a complement to culture with approximately equal diagnostic sensitivity (63 and 53% respectively) and with an acceptable specificity. IF also represents the only rapid method for the diagnosis of whooping cough at present. Serology with ELISA, measuring antibody response against the fimbrial hæmagglutinins of B. pertussis, is a new and valuable diagnostic method for retrospective confirmation of pertussis where culture and IF fail. The method is especially convenient in children since capillary blood samples give sufficient amounts of serum. The drawbacks of any one of these methods may to a large extent be overcome by their simultaneous use. However, other diagnostic methods need to be developed combining the specificity of culture, the swiftness of IF and the sensitivity of serology with ELISA.

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