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The serological diagnosis of whooping cough

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SUMMARY

Indirect haemagglutination (IHA), agglutination and complement fixation tests (CFT) for *Bordetella pertussis* antibodies were compared on paired sera from 52 suspected cases of whooping cough and single sera from 83 children with no recent history of whooping cough. All three tests detected serotype antibodies 1, 2 and 3, but the IHA test was the most sensitive; in seven cases it was the only test to show a rise in titre. It is recommended, particularly with vaccinated children, that the serological diagnosis of whooping cough should be based upon a rise in titre. There should be a gap of at least 2–4 weeks between serum samples, depending on the age and vaccination state of the child. The CFT appears to detect a different antibody from that detected by the other two tests, and in three cases it was the only test to show a rise in titre.

INTRODUCTION

Serological tests may be used to establish the diagnosis of whooping cough and antibodies to *Bord. pertussis* can be demonstrated by agglutination or complement fixation tests. Agglutination detects antibody to the heat-labile serotype antigens 1, 2 and 3 and to the heat-stable somatic antigen. A live or formalin-killed suspension is used to detect antibody to the heat-labile type antigens (Andersen, 1953). Different CFT antigens were used in two recent studies. Bradstreet *et al.* (1972) heated a bacterial suspension at 60 °C for 1 h and used the supernatant after centrifugation; the serotype of the strain used to make the antigen did not affect the serum titre, implying that the major antibody detected was to antigen 1 (common to all strains) or was not a type-specific antibody. The Combined Scottish Study (1970) used a recently isolated 1,3 strain and extracted their antigen with sodium hydroxide for 2 h at 37 °C; they do not state if type-specific antibody was demonstrated.

Indirect haemagglutination (IHA) has been used to test human sera for pertussis antibodies (Schubert, Eleff & Hermann, 1961; Soare *et al.* 1964; Adonajlo & Kozerska, 1970; Thomas, 1975). Only Schubert *et al.* tested paired sera from suspected cases of whooping cough, and none of the authors indicated which antigens attached to the red blood cells.

The IHA test described here detects type specific antibody and is compared with agglutination and a CFT.

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MATERIALS AND METHODS

Sera

The human sera tested were paired sera from 52 children aged 2 months to 14 years, with suspected whooping cough, and single sera from 83 children with no evidence of recent whooping cough (20 new-borns and 63 children aged 5 months to 13 years, with known pertussis vaccination histories). The ability of the tests to detect type specific antibodies was tested against Wellcome pertussis typing sera.

Preparation of the IHA and CFT antigen

The antigen used in the CFT and to sensitize the RBCs for the IHA test was similar to the antigen used by Bradstreet *et al.* (1972) for their CFT. A 1,2,3 strain of *Bord. pertussis* was grown for 2 days on charcoal blood agar (Oxoid CM 119) and the growth from each plate suspended in 2 ml of physiological saline. Antigen was only made if typing sera 1, 2 and 3 gave complete agglutination within 3 min. The suspension was heated in a 60 °C water bath for 1 h (shaking every 15 min) and then centrifuged at 3000 rev./min for 20 min; the supernatant, with one drop of 1% thiomersalate added to each 2 ml, was used as antigen. It could be stored for a year at 4 °C for use in the CFT but deteriorated within a month as an IHA antigen. The best way of preserving the antigen for use in both tests was by freeze-drying, when it showed no deterioration after 18 months. It could be kept for up to one year at − 20 °C, but needed restandardizing regularly for IHA.

Setting up the tests

Tanned, formolized fowl RBCs were used for the IHA test. They were formolized and sensitized in 2 or 4 ml amounts by the method of Sequeira & Eldridge (1973). The test was done in V-shaped Cooke's microtitre trays by mixing 0·02 ml volumes of each serum dilution and of sensitized RBCs, and leaving the trays at room temperature while the cells settled (about 1 h). The end-point was taken as the last well in which less than 25% of the RBCs had settled into the point of the well. The sensitized RBCs could be used immediately or on the day after they were sensitized. The agglutination test was by the method of Preston (1970), with a formolized suspension of the 1,2,3 strain. The CFT was done by the method of Bradstreet *et al.* (1972) with overnight fixation at 4 °C but with 0·02 ml volumes in U-shaped Cooke's microtitre trays. All three tests were done on each serum; the starting dilution was 1/5 and the serum was inactivated at 55 °C for ½ h before all three tests, though inactivation did not appear to affect the agglutination or IHA titres. All the titres are given as the reciprocal of the initial serum dilution as one of the tests was a CFT.

Table 1. *Reciprocal of titres of the Wellcome pertussis typing sera*

Typing serum	IHA	Agglutination	CFT
1	2560	160	40
2	1280	320	20
3	1280	160	10

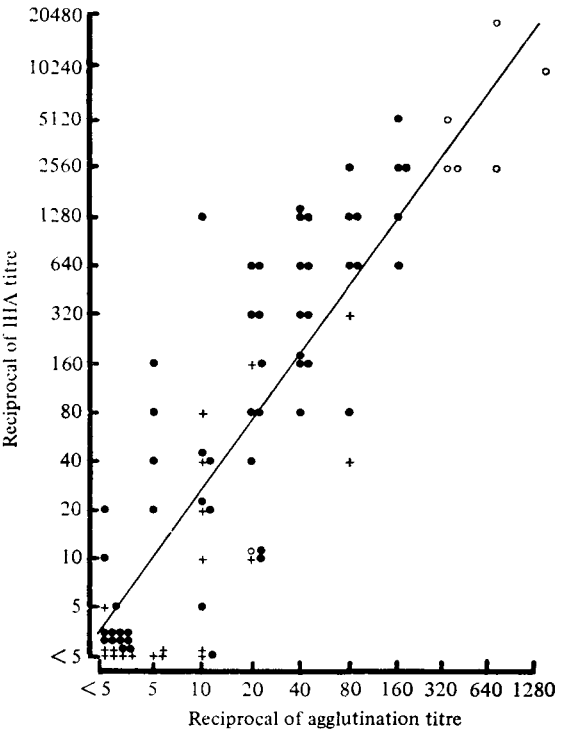

Fig. 1. Scatter diagram and regression line for single samples of sera from children with no recent history of whooping cough. ●, Children with known vaccination histories and CFT titre ⩽ 5; ○, children with known vaccination histories and CFT titre ⩾ 10; +, neonates.

RESULTS

The results of the three tests with the Wellcome typing sera is shown in Table 1. The IHA test gave the highest titres against Wellcome sera and the CFT the lowest.

There was good correlation between the IHA and the agglutination titres of the 83 sera from children with no recent history of whooping cough (Spearman rank correlation coefficient 0·88). The regression line for the IHA titres on the agglutination titres (Fig. 1) shows that the IHA titres were on average fourfold higher. The titres persisted after a dose of vaccine; IHA titres of up to 1280 and agglutination titres of up to 80 were found 5 or more years after a dose of vaccine. Six of the seven CFT-positive sera were associated with high IHA and agglutination titres, though similar titres were found in CFT-negative sera. The CFT titre did not persist long after a dose of vaccine; all the CFT titres of ⩾ 10 were

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Table 2. *Pertussis antibody titres of paired sera from children with suspected whooping cough*

Case number	Age	Weeks since onset of cough		Reciprocal of titre: Agglutination		IHA		CFT	
		1st serum	2nd serum	1st serum	2nd serum	1st serum	2nd serum	1st serum	2nd serum
				Culture-positive cases showing a rise in titre					
1†	3 months	1	9	< 5	10	< 5	10	< 5	5
2†	3 months	2	7	5	10	< 5	10	< 5	5
3†	4 years	2	5	5	20	5	80	< 5	5
4†	3 months	2	4	< 5	20	5	160	< 5	< 5
5*	11 years	3	5	160	640	2560	10240	< 5	10
6	3 years	2	5	5	160	160	5120	40	160
7	5 months	1	4	< 5	< 5	5	20	< 5	< 5
8	1 year	1	5	160	1280	2560	20480	10	40
9	1 year	1	4	< 5	< 5	40	40	< 5	10
				Culture-positive cases NOT showing a rise in titre					
10†	3 months	2	4	< 5	< 5	< 5	< 5	< 5	< 5
11†	5 months	1	4	< 5	< 5	< 5	< 5	< 5	< 5
12†	5 years	4	7	5	5	40	80	< 5	5
13	7 months	1	4	< 5	< 5	20	40	< 5	< 5
14	4 years	2	3	< 5	< 5	5	10	< 5	< 5
				Culture-negative cases showing a rise in titre					
15†	4 months	2	5	< 5	10	< 5	20	< 5	10
16†	1 year	2	8	20	80	< 5	80	< 5	20
17†	5 years	4	6	40	160	40	640	5	5
18†	1 year	2	6	< 5	5	40	640	< 5	40
19†	2 months	6	11	< 5	40	< 5	320	< 5	5
20†	10 months	1	6	< 5	< 5	< 5	< 5	10	160
21*	5 years	2	8	10	1280	20	2560	< 5	5
22*	3 years	1	2	40	160	10	160	< 5	< 5
23*	6 years	8	11	640	1280	320	2560	160	160
24*	5 years	2	3	80	320	80	2560	10	160
25	5 years	12	15	10	160	20	320	< 5	80
26†	11 months	12	16	5	10	80	320	10	10
27†	3 months	2	11	< 5	5	< 5	10	< 5	5
28†	2 months	2	6	< 5	5	< 5	10	< 5	10

Table 2 (*cont.*)

Case number	Age	Weeks since onset of cough		Reciprocal of titre: Agglutination		IHA		CFT	
		1st serum	2nd serum	1st serum	2nd serum	1st serum	2nd serum	1st serum	2nd serum
29†	7 months	2	4	< 5	5	10	320	< 5	< 5
30†	6 months	1	4	20	20	< 5	5	< 5	10
31†	3 months	2	5	< 5	5	< 5	20	< 5	< 5
		Culture-negative cases NOT showing a rise in titre							
32†	2 years	10	22	40	40	< 5	< 5	20	20
33†	2 months	3	4	10	10	20	20	< 5	< 5
34†	9 years	2	4	160	320	80	80	5	5
35†	7 months	3	4	< 5	< 5	20	40	< 5	< 5
36†	1 year	2	6	< 5	< 5	20	20	40	40
37*	4 years	6	12	80	80	320	640	< 5	< 5
38*	11 months	10	11	< 5	< 5	20	20	< 5	< 5
39*	7 years	2	5	40	40	80	160	80	80
40	14 years	5	12	640	640	10240	10240	40	40
41	5 months	4	8	80	80	640	640	5	< 5
42*	11 months	2	5	20	20	80	160	10	20
43†	3 months	1	6	10	10	10	20	< 5	< 5
44	4 years	5	8	160	160	1280	1280	< 5	< 5
45	1 year	4	7	< 5	< 5	10	10	< 5	< 5
46	5 years	3	5	20	20	320	320	< 5	< 5
47	4 months	2	6	< 5	< 5	5	10	< 5	< 5
48	2 months	3	4	< 5	< 5	20	20	< 5	< 5
49*	1 year	2	6	20	20	160	160	10	5
50	5 months	6	9	20	20	320	640	5	5
51	3 years	3	8	320	320	2560	1280	40	40
52†	5 years	5	8	< 5	< 5	10	10	< 5	< 5

* Child known to have received pertussis vaccine.
† Child known to be unvaccinated.

found in sera taken from children who had received a dose of pertussis vaccine 1–7 months previously.

When the 52 paired sera from children with suspected whooping cough were tested, 14 showed a fourfold or greater rise in titre in the agglutination test, 23 in the IHA test and 12 in the CFT (Table 2). The agglutination test never showed a greater rise in titre than the IHA test; in nine cases a rise in titre was detected by IHA but not by agglutination, but in three cases a rise was only found in the CFT.

There was considerable variation in the individual children's response to *Bord. pertussis* infection. Young or debilitated children tended to produce a very slow antibody rise; for example case 1 (Table 2) showed only fourfold rises in agglutination and IHA titres between 1 and 9 weeks after the onset of cough, and a child with leukaemia, case 25, showed no rise in antibody until 15 weeks after onset. Older or vaccinated children could produce earlier and sharper antibody rises; case 22, a 3-year-old, vaccinated as a baby, gave a 16-fold rise in IHA titre when the first serum sample was taken 1 week after the onset of cough and the second 9 days later. In such children blood taken 3–4 weeks after onset might be too late to show an antibody rise. In the culture-positive cases the average gap between the first and second sera was 3·7 weeks in the nine cases where a rise in titre was shown and 2·4 weeks in the five cases where no rise was detected.

Bradstreet *et al.* (1972) found a CFT titre of > 20 in 8 out of 618 human control sera, which suggests that a titre of > 20 might be of diagnostic significance. In only two of the 14 culture-positive cases (6 and 8, Table 2) was the CFT titre > 20 in the second serum; in both these cases there was a rise in titre in all three tests. The second serum gave a CFT titre of > 20 in 5 of the 17 cases with a rise in titre in at least one test (18, 20, 23, 24, 25); one of these five cases showed a rise in titre in IHA alone, one in the CFT alone, and three in the CFT and at least one other test. More cases were probably selected from this second group because the average time between the onset of cough and the second serum sample was $7\frac{1}{2}$ weeks compared with 5 weeks for the culture-positive group. Thus about three-quarters of the cases in these two groups would have been missed if the diagnosis had been made on a raised CFT titre. Four of the 21 cases which were both culture negative and gave no rise in titre (36, 39, 40, 51) had a CFT of > 20 in the second serum sample. In two of these cases both sera were taken 3 or more weeks after the onset of cough and it is possible that the antibody titres had already reached a plateau; in the other two cases the first serum was taken at 2 weeks which is early for plateau titres of CFT antibody to be reached, particularly in the case of the one unvaccinated child (36). The vaccination history was not known for all 52 children, but 3 of 10 vaccinated children and 3 of 11 unvaccinated children, over 9 months of age, had raised CFT titres. A raised CFT titre was not, therefore, always associated with previous vaccination.

DISCUSSION

Laboratory confirmation of whooping cough is best achieved by isolation of *Bord. pertussis*, as carriers occur rarely if at all (Linnemann, Bass & Smith, 1968). Where isolation has not been possible serological methods may be useful in individual cases and for epidemiological studies. Serology is also used to measure the response to vaccination. There is controversy about the identity of the protective antigen; Holt & Spasojevíc (1968) produced experimental evidence that the type antigens play no part in the mouse protection test (the potency test for pertussis vaccine), but Preston & Stanbridge (1972) consider that a vaccine should contain all three type antigens if it is to give good protection in children. The three tests used in this study gave titres with typing sera 1, 2 and 3, though the CFT titres were low. The children's sera were not absorbed to measure the titres of the individual type-specific antibodies, as it is an advantage if a serological diagnosis can be made on a minimum volume of serum; these three tests could be done on 0·1 ml.

The correlation coefficient of 0·88 between the IHA and agglutination titres and their persistence after vaccination suggests that these two tests usually detect the same antibody. The IHA test was more sensitive; on average it gave fourfold higher titres in vaccinated children and it was more likely to show a rise in titre in infected children. The CFT, though a less-sensitive test, detects a different antibody which does not usually persist more than 7 months after a dose of vaccine (Bradstreet *et al.* 1972, and this study). Three of the paired sera (9, 20, 30) showed a rise in the CFT only and this makes it a useful second test for diagnosis. As the IHA and the agglutination titres persist after vaccination and presumably after infection, a high titre on a single serum sample is difficult to interpret, especially in a vaccinated child. The disappearance of the CFT antibody after a dose of vaccine suggests that it could be used for diagnosis on one specimen of serum. In practice the titre rises so slowly that in this series only 2 of the 14 culture-positive cases (6 and 8) had a CFT titre of > 20 in the second serum (taken on average 5 weeks after onset). Also it seems likely that at least 1 (case 36) of the 11 children with raised CFT titres did not have pertussis infection, as culture for *Bord. pertussis* was negative and no rise in titre was detected in well-timed sera. Thus while a CFT titre of > 20 supports a diagnosis of whooping cough, a low titre does not exclude it, and false positive titres may occur. It is therefore recommended that paired sera are taken for the serological diagnosis of whooping cough.

The timing of the antibody response was variable; there was usually a gap of 2–4 weeks between sera when a rise in titre was detected, but some young or debilitated children needed a longer interval and some older, vaccinated children produced a sharp, early rise in antibody. In order to have the best chance of establishing the serological diagnosis the first serum should be taken within 2 weeks of onset of cough and the second 2–4 weeks later, depending on the age and vaccination state of the child; a further serum taken about 4 weeks after the second serum may be needed to detect a rise in titre in some children. If the

first serum is delayed till late in the disease, the results may be equivocal, particularly in older or vaccinated children when high but not rising titres are difficult to interpret.

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