

Prevalent serotypes of *Bordetella pertussis* in non-vaccinated communities

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SUMMARY

In many countries, the prevalent serotypes of *Bordetella pertussis* have changed from a mixture of types 1,2,3 and 1,2 (organisms possessing antigen 2) to a predominance of type 1,3. The timing of the change in different countries is shown to be related to the introduction of mass-vaccination with material rich in antigens 1 and 2 but weak in, or devoid of, antigen 3. In several parts of the world, there have been outbreaks of type 1,3 infection in fully vaccinated children.

Non-vaccinated communities in various parts of the world still show the pattern of serotypes which existed elsewhere before mass-vaccination.

In order to avoid the disappointments experienced in the past, it is essential that pertussis vaccine for use in previously non-vaccinated communities, like that for any other country, should be rich in each of the three antigens.

INTRODUCTION

For some years it has been realized that the prevalent strains of *Bordetella pertussis* in several countries have changed from a predominance of those possessing antigen 2 (types 1,2,3 and 1,2) to a predominance of type 1,3. Preston (1965*a*, 1966) attributed this change, in Britain, to a lack of antigen 3 in the vaccine, resulting in selection of resistant bacteria in an inadequately immunized population; and later evidence (Preston, 1970*a*) pointed to a similar explanation for other countries also.

Cohen, Hannik & Nagel (1971) rejected this hypothesis and suggested that the poor protection given by pertussis vaccine in Britain was unique: 'similar changes in the agglutinogenic pattern of circulating strains' were consistent with good protection and had been reported from 'many countries where the morbidity rate showed a steeper decline than in Britain'. Likewise rejecting the hypothesis, Aftandeliants & Connor (1974) reported a similar prevalence of type 1,3 strains more recently in the U.S.A. – the same ratio of serotypes as 'from other parts of the world', though they mentioned only Britain, Canada and Russia.

However, Cohen *et al.* gave no evidence that vaccines made outside Britain were either rich in antigen 3 or giving good protection, and Aftandeliants & Connor admitted that they had not studied the antigenic composition of the vaccines with which a third of their cases of whooping-cough had been 'immunized'.

If the world-wide change of prevalent serotypes is not due to vaccination, it might be expected to have occurred also in non-vaccinated communities; but these are usually areas with inadequate facilities for culturing the organism, though by no means lacking in whooping-cough patients.

After much initial frustration, a significant number of strains has been collected from such areas, and the present report compares them with strains isolated from vaccinated communities.

MATERIALS AND METHODS

Strains of Bordetella pertussis

Several hundred strains isolated from cases of whooping-cough have been gratefully received from five continents.

Most of these have come from laboratories in different parts of Britain. Details of the sources may be found elsewhere (Preston, 1963, 1965*a*, 1976).

Strains have kindly been sent by workers in many other countries also, as follows: Argentina (Dr Elena E. Duhart, National Institute of Microbiology, Buenos Aires), Australia (Dr J. Gulasekharan, Commonwealth Serum Laboratories, Parkville), Canada (Dr A. J. Wort, Izaak Walton Killam Hospital for Children, Halifax), India (Dr K. C. Agarwal, Postgraduate Institute of Medical Education and Research, Chandigarh), Israel (Prof. W. Hirsch and Dr M. Shmilowitz, Kupat-Holim Central Laboratory, Haifa), Kenya (per Dr H. Dikken and Miss G. S. Lighthart, Royal Tropical Institute, Amsterdam, Holland), Malaysia (Dr Thong Mee Len, University of Malaya, Kuala Lumpur), Poland (Dr T. Walter, Sanitary Epidemiology Station, Poznan), U.S.A. (Dr J. W. Bass, Tulane University, New Orleans), Yugoslavia (Dr Vera Spasojevic, Torlak Institute for Sera and Vaccines, Belgrade).

Serotyping of pertussis strains

Full details of the methods have been described previously (Preston, 1970*b*; Stanbridge & Preston, 1974*a*). Until about a year ago, tests were done here with monospecific sera produced in this Department. Since then, use has been made of sera kindly supplied by Prof. M. S. Zakharova from the World Health Organization reference laboratory at the Gamaleya Institute of Epidemiology and Microbiology in Moscow, after preliminary tests with selected strains of different serotypes had shown that the same results were obtained with her sera and ours.

Many of the strains isolated recently in Kenya have been typed independently in four laboratories in different countries (Miss G. S. Lighthart, personal communication), and the four sets of results are almost identical. In view of the known variation to which serotypes of *Bord. pertussis* are prone (Stanbridge & Preston, 1974*a*), these findings are of great significance and suggest that wide differences in typing data are unlikely to result from variation occurring during a moderate number of subcultures of the strains after isolation. Full details of the typing of the Kenyan strains will form part of a joint report which the various participants intend to publish.

Table 1. *Serotypes of Bordetella pertussis several years after the introduction of mass-vaccination*

Country of origin of cultures	Date of isolation	Source of information	No. of isolates of each serotype			
			1,2,3†	1,2	1,3‡	Total
Australia	1962-6	*	4	1	18 (78%)	23
U.S.A.	1966	*	0	0	4 (100%)	4
	1970-3	Aftandelians & Connor (1974)	5	0	30 (86%)	35
Poland	1966-8	*	2	0	24 (92%)	26
Israel	1967-71	*	4	1	56 (92%)	61
Canada	1963-4	Chalvardjian (1965)	1	1	56 (97%)	58
	1971	*	0	0	6 (100%)	6
Britain	1960-3	*	6	0	19 (76%)	25
	1963-4	*	21	2	132 (85%)	155
	1974-5	*	80	11	333 (79%)	424

* Strains received by the author in Manchester and serotyped by him.

† A few of these cultures were mixtures of two or more of the serotypes: 1,2,3; 1,2; 1,3.

‡ A few of these cultures were mixtures of types 1,3 and 1.

Table 2. *Serotypes of Bordetella pertussis before the introduction of mass-vaccination*

Country of origin of cultures	Date of isolation	Source of information	No. of isolates of each serotype			
			1,2,3†	1,2	1,3‡	Total
Denmark	1950	Andersen (1953)	31	14	10 (18%)	55
Britain	pre-1958	*	4	8	1 (8%)	13
	pre-1958	Lacey (1960)	18	5	2 (8%)	25
Australia	1950-9	Blaskett <i>et al.</i> (1971)	8	30	3 (7%)	41
Yugoslavia	1954-7	*	3	3	1 (14%)	7
Argentina	1966-9	*	1	2	1 (25%)	4
India	1971-4	*	3	1	1 (20%)	5
Malaysia	1972-4	*	3	0	1 (25%)	4
Kenya	1974-5	*	12	13	9 (26%)	34

* † ‡ See footnotes to Table 1.

RESULTS AND DISCUSSION

Comparison of pertussis serotypes in vaccinated and non-vaccinated communities

Table 1 provides ample evidence, from countries widespread over four continents, to support the impression that the prevalent serotype of *Bord. pertussis* (more than 75% of isolates) has been type 1,3 in recent years. This applies to samples taken during the last 15-years and, in each case, taken several years after the introduction of mass-vaccination.

In contrast, Table 2 shows that type 1,3 strains constituted only a small proportion of isolates in earlier years, before mass-vaccination. Moreover, this

ratio applies even in recent years to countries, again spread over three continents, where mass-vaccination has not yet been introduced (Argentina, India, Malaysia, Kenya). In non-vaccinated communities, the majority of isolates were, and still are, strains possessing antigen 2 – that is, either type 1,2,3 or type 1,2 (these two types occurring in approximately equal proportions).

Influence of vaccine composition on change of serotype

In the 1950s and early 1960s (Preston, 1965*a*), British vaccines contained strains that had been prevalent in pre-vaccination days. They were rich in antigens 1 and 2 but often lacking in antigen 3. Consequently, they were highly effective in preventing infection by organisms possessing antigens 1 and 2 (types 1,2 and 1,2,3), and they therefore confined these infections to non-vaccinated individuals. But they did not give adequate protection against type 1,3 strains – organisms which are relatively weak in antigen 1 but rich in antigen 3, and which therefore had a selective advantage in children whose vaccination raised insufficient antibody 3.

Cohen (1970) suggested that this explanation may be invalid, because a similar change of prevalent serotype had occurred in Holland, although strains possessing all three antigens had been used in the manufacture of Dutch vaccine.

However, it had already been noted (Preston, 1965*b*) that the crucial data about vaccines are not so much the serotypes of the strains from which they are said to have been made, as the antigens detectable in the final blend. Moreover, British vaccines were not alone in being suspect. Dr E. K. Andersen (personal communication) wrote from Denmark in 1965: 'I have just tested ten of our pertussis vaccines, and to my great surprise I was unable to demonstrate the agglutinogenic factor 3 in them, although we use strains containing all the known antigens for preparation of our vaccines.'

From Poland, where vaccination was introduced in 1960 (Walter, 1968), came information of a similar situation to that in Britain. By 1966–8, type 1,3 strains predominated (Table 1), and many of these were from children who had received a full course of pertussis vaccine; but five out of six batches of Polish vaccine, tested here in Manchester at that time, were found to contain only antigens 1 and 2, not 3.

The time-relation between vaccination of the population and the change of prevalent serotypes can be seen in studies from the U.S.A. where mass-vaccination was introduced about 1950. Eldering, Holwerda, Davis & Baker (1969) note that only 13 (28%) of 46 strains isolated in 1938–45 were of type 1,3; but, over the period 1951–5, the proportion had already increased to 77%; and by 1966–7 it was 84%.

Similar changes occurred later in the U.S.S.R. corresponding to later introduction of pertussis vaccine. In Ryazan, where vaccination had reached over 80% of the child population in 1964, the proportion of type 1,3 isolations changed from 5% in 1964, and 9% in 1965, to 59% in 1966, by which time the majority of isolates were from children who had been fully vaccinated – with at least three doses (Demina, Larina & Devyatkina, 1968). These authors also noted, from an

earlier study of cases in Moscow, that type 1,3 strains had not been encountered in 1948–56 ('before the introduction of mass anti-pertussis vaccination'), but they occurred in 16% of cases in 1960–1 ('2–3 years after the start of vaccination'). Later, Kuznetsov (1972) found that 59% of his isolates in Moscow in 1967 were of type 1,3; and he noted other similarities to the situation in Britain: vaccines were prepared chiefly from type 1,2 strains (46 of 57 batches), and isolates from fully vaccinated children were predominantly (70%) of type 1,3, whereas isolates from non-vaccinated children were predominantly (76%) of type 1,2,3.

From Australia (Blaskett, Gulasekharan & Fulton, 1971) came a warning, that type 1,3 vaccine alone is inadequate also. As in other countries, their early vaccines had a low content of antigen 3, though rich in antigen 2; and by 1961 (three years after vaccination began) the prevalent serotype in the community had changed from type 1,2 to type 1,3. In 1962, the previous vaccine strains (of type 1,2) were replaced by fresh isolates (of type 1,3) and, within two years, there was an upsurge of isolates possessing antigen 2, and these organisms infected even children who had received the type 1,3 vaccine.

Thus Britain was by no means unique in finding that the prevalent serotype in the community was determined by the composition of the vaccine.

Efficacy of pertussis vaccine

The question now arises as to whether it is possible to produce a pertussis vaccine which will protect against *all* serotypes of the organism.

The ineffectiveness of much British vaccine, manufactured before 1968, was clearly shown in a survey by the Public Health Laboratory Service (1969); but, from that time, antigen 3 has been deliberately incorporated in British vaccine (Perkins, 1969) and all three antigens are detectable in the final blend (Preston, *et al.* 1974). Evidence is now appearing (Preston, 1976) which indicates that current British vaccine is once again highly effective, but the efficacy now covers all serotypes and not merely strains possessing antigen 2.

The British experience of inadequate vaccine in the 1960s, however, must not be considered as unique. Complacency about the efficacy of vaccine in other countries, at that time, was not necessarily well founded. Examples of concern about pertussis infection occurring in vaccinated children have been quoted above, from Poland, the U.S.S.R. and Australia.

Similar deficiencies of pertussis vaccine have been reported also from Israel (Shmilovitz, Preston, Zaltser & Cahana, 1972; Preston, 1973) and Canada (Chalvardjian, 1965). In both countries, type 1,3 infections were occurring in fully vaccinated children, and there was evidence that a high proportion of vaccine was weak in, or devoid of, antigen 3. Moreover, Dr A. J. Wort (personal communication) found reason to doubt the popular view in Nova Scotia that whooping-cough was caused by adenovirus, when in 1971–2 he isolated over 60 pertussis cultures of type 1,3.

Evidence that all was not well in the U.S.A. came from Dr J. W. Bass (personal communication) who wrote from Tulane University in 1966: 'Many of the cases coming to our attention (some 20–30) had histories of adequate primary and booster

immunization against pertussis.' More recently, from San Diego (Aftandelians & Connor, 1974), came a report that a third of their series of culture-positive cases of whooping-cough in 1970-3 had been 'immunized', some of the patients developing a pertussis infection within nine months of receiving 'a complete series of three or more DPT injections'. They continue: 'The reason for immunization failure in these instances is unknown', but surprisingly: 'no attempt was made to perform serotyping of the different vaccines' that these patients had received! What was unique in Britain was not so much the nature of the situation as the thoroughness with which it was studied.

Guidance for immunization of non-vaccinated communities

In countries which have introduced mass-vaccination, the prevalent serotypes of *Bord. pertussis* have changed from a mixture of types 1,2,3 and 1,2 (organisms possessing antigen 2) to a predominance of type 1,3. Evidence given above indicates that this change has been encouraged by the use of vaccines rich in antigens 1 and 2 but weak in, or devoid of, antigen 3.

This reasoning is not actually in conflict with the views of Cohen (1970) who claimed that a similar change of serotype may occur even when all three antigens were incorporated in the vaccine. The problems are, first, that factor 3 appears to be the least antigenic of the three factors (Preston *et al.* 1974; Stanbridge & Preston, 1974*b*), and secondly, that it may be lost either phenotypically or by mutation (Stanbridge & Preston, 1974*a*) during the manufacture of an individual batch of vaccine from satisfactory stock cultures. Manufacturers are now recommended to include all three antigens in their vaccine, but it is essential for them to ensure that, *after manufacture*, each batch is indeed rich in all three (Preston & Stanbridge, 1976; Agarwal & Preston, 1976).

If these conditions were applied to vaccine which is now to be used in previously non-vaccinated communities, the setbacks which have occurred in other countries might be avoided, and a more rapid and more permanent decline in the incidence of whooping-cough could be expected.

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