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The serotypes of *Bordetella pertussis* isolated in Great Britain between 1941 and 1968 and a comparison with the serotypes observed in other countries over this period

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SUMMARY

Classification, by agglutinogens, of 634 isolates of *Bordetella pertussis* collected from 1941 to 1968 in Great Britain demonstrated that a change from a predominantly 1,2,0,4 serotype (75% of those examined during 1941–4) to a predominantly 1,0,3,0 serotype (73% of those examined during 1966–8) occurred sometime after 1953. Furthermore, evidence from the examination of isolates collected between 1941 and 1953 suggests that the change may have been gradual. Isolates of serotype 1,2,3,4 made up 20–30% of the total of our cross-country selection for the periods 1941–4, 1946–9, 1950–3 and 1966–8, but over shorter periods in individual areas the percentage varied from negligible to as high as half of those isolated. Results from other countries show a similar drift towards a 1,0,3 serotype but more often from a 1,2,3 than from 1,2,0 serotype.

The value, in epidemiological studies, of extended information obtained by monospecific typing sera to all six, rather than only two or three agglutinogens, and confirmation of the results by agglutinin production is demonstrated: for instance not all 1,0,3 isolates were identical.

INTRODUCTION

The suggested change over two decades of the serotype (based on possession of agglutinogens) of *Bordetella pertussis* is now well documented (Preston, 1963, 1965). The result of the Public Health Laboratory Service (PHLS) investigation (1973) has confirmed the large numbers of organisms of 1,0,3 serotype isolated in the period 1966–8 but the information about earlier isolates was rather limited. Many isolates of *B. pertussis* collected in Great Britain between 1941 and 1953, however, had been sent to the Lister Institute, Elstree, and dried after a minimum number of subcultures after isolation. We have compared 179 of these with 455 sent to us between 1966 and 1968. The results are presented here together with our experiences in serotyping and with possible future implications of this work.

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METHODS

Isolates

Isolates were stored in the lyophilized state. One hundred and seventy-nine were collected between 1941 and 1953 in Belfast, Cardiff, Leeds, North London (Colindale) and Oxford; 455 were collected over a wide area of Britain during the PHLS survey between November 1966 and April 1968 and were received from the (then) Medical Research Council Laboratories, Hampstead, from The Wellcome Research Laboratories, Beckenham, and from the Public Health Laboratories in Manchester and Liverpool.

Isolates from the PHLS survey were collected as stated (Public Health Laboratory Service, 1969) and it is likely that the same method was used for the older isolates. All isolates received at the Lister Institute on charcoal agar or Bordet-Gengou (BG) medium were subcultured twice on BG medium before being serotyped and dried either in 10% dried milk or in plasma containing 7% glucose, and stored in the lyophilized state under vacuum.

Expression of serotype

Any agglutinogen tested for is denoted by its number, if present, and by 0 if it is absent. Thus a 1,2 strain becomes 1,2,0 when tested for agglutinogens 1–3, 1,2,0,4 when tested for agglutinogens 1–4 and 1,2,0,4,0,0 when tested for agglutinogens 1–6. Agglutinins are similarly represented.

Preparation of monospecific agglutinating sera from rabbits

Strains of known serotype, most of which are now available from the National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, were used to make monospecific typing sera. Some were obtained from Dr E. K. Andersen (3747, NCTC 10908; 3865, NCTC 10906; 2 atox.) and Dr M. Haire (B16, NCTC 10907). Other strains (D35726, NCTC 10909 and D41633, NCTC 10910) were collected during the PHLS survey 1966–8. A phase I variant strain (NCTC 10905) possessing only agglutinogen 1 which had been derived from strain 3747 (Dolby & Bronne-Shanbury, 1975) and a similarly produced variant of strain 2 atox. possessing agglutinogens 1 and 2 were used also.

The agglutinating sera were made in rabbits, previously shown to be free of *B. bronchiseptica*, by intravenous inoculation of a vaccine made from a strain of known serotype as described previously for mice (Bronne-Shanbury, 1976). (A sufficiently high agglutinin titre of the sera was obtained using 5–8 injections of 0·2–0·5 ml. of vaccine at 10×10^9 organisms/ml.) The rabbits were bled 10–14 days after the last injection and the sera were selectively absorbed using formalin killed or autoclaved suspensions of strains of known serotype as shown in Table 1. Better typing sera were produced by absorbing 1–2 ml. volumes rather than 5–10 ml. The dilution at which the absorbed sera could be used for routine serotyping was determined by titrating with strains of known serotype and the maximum agglutinating dilution was taken as one half of the observed end-point dilution.

Agglutinin 1. Since all phase I strains so far tested were found to contain

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Table 1. *Scheme for the preparation of monospecific agglutinating sera for* Bordetella pertussis

Specificity of prepared anti-serum for agglutinogen	Strain used to elicit antiserum	Strain used as absorbing suspension	Agglutinins removed by absorption
1	3747 variant (1,0,0,0,0,0)*	Autoclaved Homologous	None†
2	3865 (1,2,0,4,0,0)	D41633 (1,(2)‡, 3,4,0,0)	1 and 4, some 2
	2 Atox variant (1,2,0,0,0,0)§	B16 (1,0,3,0,0,0)	1
3	B16 (1,0,3,0,0,0)	3747 variant (1,0,0,0,0,0)	1
4	3865 (1,2,0,4,0,0)	3747 variant (1,0,0,0,0,0)	1
		3747 (1,2,0,0,5,6)	2
5	3747 (1,2,0,0,5,6)	3865 (1,2,0,4,0,0)	1 and 2
		D35726 (1,0,3,0,0,(6))	6
6	D35726 (1,0,3,0,0,6)	B16 (1,0,3,0,0,0)	3

* Serotype.
† Agglutinins to heat-stable agglutinogens only removed.
‡ Weak agglutinogen.
§ Not available from National Collection.

agglutinogen 1, antisera containing only agglutinin 1 were made with a strain containing no other heat-labile agglutinogen so that absorption with autoclaved suspensions of phase I strains was sufficient.

The strain usually used in most laboratories, Gl 353z, was found both by Holt (1968) and at the Lister Institute to elicit agglutinins to at least agglutinogens 2 and 3 in addition to 1. We therefore used the spheroplast-derived 3747 variant possessing only agglutinogen 1. Agglutinins to the heat-stable O-agglutinogens which would normally be removed during the course of selective absorption, were absorbed with an autoclaved suspension of the homologous or any other phase I strain.

Agglutinin 2. The 2 atox. variant was used as a vaccine as less absorption was required and the titre of the resultant serum was higher. Similar selection of suitable variants could make the preparation of other sera easier. Alternatively, serum was prepared using 3865 with careful absorption as shown in Table 1.

Agglutinins 3 *and* 4. High-titre sera were easily obtained using the scheme shown in Table 1. In the preparation of agglutinin 3, continued vaccination of rabbits with B16 sometimes produced traces of agglutinin 6, which could readily be absorbed with strain 3747.

Agglutinin 5. Strains containing agglutinogen 5 are extremely rare. In the strain used for the production of this agglutinin it is found associated with agglutinogen 6, which only agglutinates weakly and does not always show on direct serotyping. The serum must therefore be made with a sufficiently high titre for traces of unabsorbed agglutinin 6 to be diluted out.

Agglutinin 6. Agglutinogen 6 is very weak and is closely associated with agglutinogen 3 (Eldering, Holwerda & Baker, 1967). Some or all agglutinin 6 is therefore absorbed during the absorption of agglutinin 3 and it is extremely difficult to make monospecific agglutinating serum of a sufficiently high titre for use in routine testing. Of the seven antisera made in various ways only two could be used. One method is shown in Table 1.

Determination of serotype

All isolates from the PHLS survey were typed before they were preserved. Subsequent work on these isolates after lyophilization, storage and reconstitution showed that there were no changes in serotype. The older lyophilized isolates were reconstituted and tested at the same time. Agglutinogens were tested for (i) by direct agglutination in microtitre trays and (ii) by agglutinin production in mice and assays of the agglutinins in microtitre trays.

Direct agglutination. The micro-method of agglutination was used. Living suspensions of 18–24 hr. cultures were emulsified in phosphate buffered saline pH 7·4 and adjusted to 10×10^9 organisms/ml. by opacity. Monospecific typing sera were diluted as described above and pipetted in 0·02 ml. volumes from '50 dropping pipettes' into the wells of plastic microtitre trays (Disposo, Linbro Chemical Co. Inc., 681 Dixewell Ave., New Haven, Conn. 06511, U.S.A.). Unabsorbed rabbit serum known to be free from agglutinins to *B. bronchiseptica* was used at the same dilutions as the typing sera as a control of the auto-agglutinability of each suspension. The suspensions of the isolates under test and control suspensions of strains of known serotype were added. Each tray was covered with a glass tile and incubated at 56° C. for 1 hr. Readings were made with the aid of a plate microscope and overhead lighting after the covered trays had stood at room temperature overnight. Results were recorded as + +, +, ±, tr and – indicating the degree of agglutination. Each test was repeated at least twice.

Agglutinin production in mice. Where results of direct agglutination varied from day to day and subculture to subculture, agglutinins were produced in mice and the sera were analysed as described elsewhere (Bronne-Shanbury, 1976).

RESULTS AND DISCUSSION

The cultures which form the basis for this paper were all received in our laboratory very soon after isolation from patients. We have called them 'isolates', since in an epidemic, one 'strain' may recur many times in patients in one small area. The influence on the serotyping results of the number of isolates in an epidemic has not been considered here. The exact nature of each isolate is unknown, that is whether one or more colonies were initially cultured from each swab and how

Table 2. *Information about the serotype of isolates obtained by using all six monospecific agglutinating sera, instead of only* 1, 2 *and* 3

Typing with agglutinogens 1–3		Typing with agglutinogens 1–6 (full typing)	
Type	No. of isolates	Type	No. of isolates
1,2,0	19	1,2,0,0,0,0	1
		1,2,0,4,0,0	17
		1,2,0,0,5,6	1
1,0,3	205	1,0,3,0,0,0	183
		1,0,3,0,0,6	21
		1,0,3,4,0,0	1
1,2,3	65	1,2,3,0,0,6	2
		1,2,3,4,0,0	52
		1,2,3,4,0,6	9
		1,2,3,4,5,0	1
		1,2,3,0,5,0,	1

Table 3. *Information about the serotype of isolates* obtained by using agglutinin production as well as direct agglutination*

Serotype results by direct agglutination	No. isolates	Serotype by agglutinins produced	No. isolates
1,0,3,4,0,0	4	1,2,3,4,0,6	2
		1,2,3,4,0,0	2
1.0,0,0,0,0	7	1,2,0,0,0,0	3
		1,2,0,4,0,0	2
		1,2,3,0,0,0	1
		1,0,3,0,0,0	1
1,0,0,4,0,0	2	1,2,0,4,0,0	2
1,2,0,0,0,0	1	1,2,3,4,0,0	1
1,0,3,0,0,0	1	1,2,3,0,5,0	1

* Examples are given here for isolates for which extended information was obtained by using agglutinin production. Implications of using both methods are dealt with at length elsewhere (Bronne-Shanbury & Dolby, 1976).

consistently any particular subculturing practice was carried out. Preston & Stanbridge (1972) have drawn attention to sampling procedures and this is very important in considering the occurrence of more than one serotype in human and animal infections. The heterogeneity of strain 18–323 which had been mouse passaged (Cameron, 1967) and of a 1,2,3 strain recovered from intranasal infections in marmosets (Stanbridge & Preston, 1974) has been reported.

Use of six monospecific typing sera

In routine typing, to provide simple information, it is obviously impracticable and unnecessary to use tediously prepared and carefully checked antisera highly specific for all six heat-labile agglutinogens of *B. pertussis*. For extended studies,

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Table 4(*a*). *Analysis of serotypes isolated in British towns in* 1941–53 *and* 1966–8 (*direct serotyping and agglutinin production*)

Town of origin	No. of isolates	No. of isolates of serotype					
		1,2,0,0	1,2,0,4	1,2,3,0	1,2,3,4	1,0,3,0	1,0,3,4
		I. 1941–53					
Cardiff	44	—	33	—	11	—	—
N. London	41	2	33	—	5	1	—
Oxford	39	—	20	—	12	7	—
Leeds	23	—	11	—	11	1	—
Manchester	11	—	5	—	5	1	—
		II. 1966–8					
Bradford	22	—	—	—	4	18	—
N. London	56	—	—	1	6	49	—
Coventry	44	—	1	—	14	28	1
Dundee	55	—	—	—	10	45	—
Edinburgh*	27	—	—	—	—	27	—
Glasgow	47	—	—	—	4	43	—
Manchester	89	—	1	—	32	56	—

* All isolated between January and July 1967, therefore probably one epidemic.

Table 4(*b*). *Detailed analysis,* by year, of the three main serotypes of* B. pertussis *in Manchester and North London,* 1946–67 (*direct serotyping and agglutinin production*)

	Total isolates	1,2,0,4	1,2,3,4	1,0,3,0
Manchester				
1947	2	1	1	—
1949	2	1	1	—
1950	7	3	4	—
1967	89	1	32	56
North London				
1946	6	6	—	—
1947	3	3	—	—
1948	8	6	2	—
1949	10	8	1	1
1953	12	10	2	—
1967	48	—	6	42

* See Table 4(*a*).

however, such sera are essential and about half of our isolates were typed for agglutinogens from 1 to 6. Table 2 shows a comparison of the agglutinogens of 289 isolates as obtained by 1–3 and 1–6 serotyping. The 1,2,0 group was the most homogeneous although not completely so, with agglutinogens 2 and 4 occurring together; the 1,0,3 group varied in the absence or presence of agglutinogen 6, a common associate of agglutinogen 3. The 1,2,3 group was the most heterogeneous.

Assuming that agglutinogen 1 is always present in naturally occurring strains of phase I organisms (Andersen, 1952, 1953) there are 32 possible combinations of serotype from 1,0,0,0,0,0 to 1,2,3,4,5,6. We have found only 12 of these; 11 are

Table 5. *Serotypes isolated between* 1941 *and* 1968 *in the Great Britain*

Serotype	1941–4 (% total)	1946–9 (% total)	1950–3 (% total)	1966–8 (% total)
1,0,0,0	0	0	0	0
1,2,0,0	0	4	0	0
1,2,0,4	75	67	60	1
1,2,3,0	0	0	0	1
1,2,3,4	21	25	32	24
1,0,3,0	4	4	8	73
1,0,3,4	0	0	0	2
Total isolates tested	28	51	100	455

shown in Table 2 and 1,0,0,0,0,0 (Table 3) makes the twelfth. The geographical origin of strains collected in two periods, one of 12 and one of 2 years is given in Table 4(*a*). The chronological origin of isolates collected during 4 years in Manchester and 6 years in North London are given in Table 4(*b*). The value of accurate serotype determination in epidemiological investigations is very evident from these tables, in which group 1,2,0 becomes subdivided into 1,2,0,0 and 1,2,0,4 and group 1,2,3 into 1,2,3,0; 1,2,3,4; and 1,0,3,4 (the latter because it was originally erroneously typed by commonly used 2 serum made against a 1,2,0,4 strain without absorption of agglutinin 4). The infection in Edinburgh was the only 'pure' one. Typing in this extended way is necessary for identification of infecting strains in any area and detection of any subsequent change.

Use of agglutinin production in serotyping

This has been found a sensitive way of detecting agglutinogens (Bronne-Shanbury, 1976). For most strains of serotype 1,2,0,4 and 1,0,3,0 no difference was found in the results by direct typing and agglutinin production (Bronne-Shanbury & Dolby, 1976). Table 3 illustrates additional information about serotype gained by agglutinin production for some isolates which were difficult to type by direct agglutination.

Use of agglutinin production is of most value in investigating strains difficult to type directly and in answering questions about possible changes of serotype *in vivo*; this has been dealt with elsewhere (Bronne-Shanbury & Dolby, 1976). It is to be noted that agglutinogen 4 is sometimes undetected by agglutinin production despite a positive agglutination when typed directly, a phenomenon attributable to its low antigenicity in mice.

Serotypes of B. pertussis *isolated in Great Britain* 1941–68

Of the 634 isolates investigated 245 were typed directly for agglutinogens 1–6 using our monospecific antisera and 37 were analysed for 1–6 by agglutinin production; the rest (352) were typed for 1–4 using our monospecific antisera. The results are shown in Table 5. The change from 1,2,0,4 to 1,0,3,0 is obvious but the fairly constant number of 1,2,3,4 is interesting. The change in predominant sero-

Table 6(*a*). *Comparison of the most predominant serotypes in various countries (expressed as % total)*

	Great Britain				U.S.A.*			Victoria, Australia†			Holland‡			Denmark§			
Year	1,2,3,4	1,2,3,0	1,2,0,4	1,0,3,0	1,2,3	1,2,0	1,0,3	1,2,3	1,2,0	1,0,3	1,2,3	1,2,0	1,0,3	1,2,3,4	1,2,3,0	1,2,0,4	1,0,3,0
1946	.	.	.	.													
1947	.	.	.	.													
	25	0	67	4													
1948	.	.	.	.	50	8	40										
1949	.	.	.	.													
1950	.	.	.	.	.	.	.	.	.	.	56	54	0				
1951	.	.	.	.	.	.	.	.	.	.	.	.	.				
	32	0	60	8										16	47	5	3
1952	.	.	.	.	.	.	.	.	.	.	.	.	.				
					19	3	77										
1953	.	.	.	.	.	.	.	.	.	.	.	.	.				
1954	.	.	.	.	.	.	.										
								20	73	7							
1955	.	.	.	.	.	.	.										
1956	.	.	.	.	.	.	.										
1957	.	.	.	.	.	.	.										
					6	2	92										
1958	.	.	.	.	.	.	.	.	.	.	4	4	76				
1959	.	.	.	.	.	.	.										
1960	.	.	.	.	.	.	.										
1961	.	.	.	.	.	.	.										
1962	.	.	.	.	.	.	.	20	8	72	.	.	.				
1963	.	.	.	.	3	0	92	.	.	.	.	.	.				
														43	0	3	48
1964	.	.	.	.	.	.	.	.	.	.	.	.	.				
											6	12	53				
1965	.	.	.	.	.	.	.	.	.	.	.	.	.				
1966	.	.	.	.	.	.	.										
1967	24	1	1	73	0	2	96	24	12	74							
1968	.	.	.	.	.	.	.										
1969	.	.	.	.	.	.	.										

* Eldering, Holwerda & Baker (1969). † Blaskett, Gulasekharam & Fulton (1971). ‡ Cohen (1970). § Anderson (1953, 1966).

Table 6(*b*). *Comparison of the most predominant serotypes in small areas* (*expressed as* % *total*)

Year	Toronto Hosp., Canada*			Ryazan U.S.S.R.†			Estonia U.S.S.R.‡			East Berlin U.D.D.R.§			Leeds G.B.‖			Manchester G.B.¶		
	1,2,3	1,2,0	1,0,3	1,2,3	1,2,0	1,0,3	1,2,3	1,2,0	1,0,3	1,2,3	1,2,0	1,0,3	1,2,3	1,2,0	1,0,3	1,2,3	1,2,0	1,0,3
1960	.	.	.	.	.	.	.	.	.	.	.	.	1	0	99			
1961	.	.	.	.	.	.	.	.	.									
1962	.	.	.	.	.	.	.	.	.									
1963 **										50	49	0						
1963	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
1964	1	1	56	90	5	5	.	.	.	.	.	.	.	.	.	15	0	85
1965	.	.	.	84	7	9	.	.	.									
1966	.	.	.	31	10	57	.	.	.	60	33	7						
1967	.	.	.	.	.	.	0	0	27									
1968	.	.	.	.	.	.	0	0	90									

* Chalvardjian (1965).
† Demina, Larina & Devyatkina (1968).
‡ Maixnerova, Burianova-Vysoka & Stansky (1972).
§ Mebel (1968).
‖ Preston (1963).
¶ Preston (1965).
** These figures from Toronto are for August 1963–September 1964 only.

type could have been gradual rather than sudden but such a conclusion could be reached only by investigation of samples more carefully chosen than ours and over the whole period of nearly 30 years. Unfortunately we have insufficient details to show a month-by-month picture of infection by different serotypes, and therefore possible epidemics, but a year by year picture is shown in Table 4(*b*). It would appear that the 'pure' infection in Edinburgh shown in Table 4(*a*) is the result of infection with a single strain. If more than one epidemic strain were present in an area at the same time, then a child might possibly be infected with more than one serotype, e.g. Manchester 1950, 1967.

A comparison of the British results was made with those available from other countries: these results are summarized, with references, in Tables 6(*a*) and (*b*), for large and small areas respectively. A change in the most frequently isolated serotype has occurred in nearly every area considered. The time of the serotype change varies throughout Europe, that in the U.S.S.R. being the most recent change. This change had occurred in Britain by 1966 (it may have occurred by 1960, see Table 6(*b*), Leeds), in the U.S.A. by 1951, in Victoria, Australia, by 1960, in Holland by 1956, in Denmark by 1962 and in Toronto, Canada by 1963. In the U.S.S.R. changes occurred in 1966 in Ryazan and in 1968 in Estonia. The dominant serotype in all areas except East Berlin appears to be 1,0,3, including Czechoslovakia where this has been so since 1963. However, in East Berlin the predominant serotype has remained 1,2,3 since 1962.

One suggested reason for the change in serotype is that of vaccination by strains containing only some agglutinogens (Preston, 1963, 1965) but this is perhaps not borne out by the results in Denmark where 1,2,3 was predominant and presumably originally used in vaccine strains and yet the change to serotype 1,0,3 also occurred. Virulence and serotype are not related but the emergence of a virulent strain of one particular serotype could produce world colonization.

Careful epidemiological studies using more sensitive serotyping might reveal possible reasons for the emergence of predominating serotypes.

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