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Bordetella pertussis Serotypes in the United States

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To determine the *Bordetella pertussis* serotypes currently causing whooping cough in the United States, recently isolated cultures from different geographic areas were studied. Specific adsorbed antisera were prepared in our laboratory and used in both tube and slide agglutination tests. Among the 177 cultures isolated during 1966 and 1967 in seven states and one Canadian province, the predominant serotype was 1.3 (or 1.3.6), represented by 85% of the cultures. Fifteen cultures were serotype 1.2.3.4.6. The 376 cultures isolated in the Grand Rapids area during the last 30 years showed a changing pattern from serotype 1.2.3 during the early years to the currently prevailing 1.3 (or 1.3.6). During the 2-year study period, seven cultures of *B. parapertussis* were isolated in Michigan, and seven were received from other states. One culture of *B. bronchiseptica*, recovered from a child in Boston, was sent to us.

New interest in *Bordetella pertussis* serotypes developed after reports appeared by Preston and Evans (12) and Preston (9, 10, 11) linking serotype with protection. According to these workers, the type-specific factors (agglutinogens) 2 and 3, as well as the species-specific factor 1, are associated with immunity to pertussis infection in man. Preston concluded that vaccine prepared from *B. pertussis* cultures lacking factor 3 would not protect as well against infection with factor 3 cultures as would a vaccine which includes this antigen. In England, according to his reports, the serotype of cultures causing whooping cough has changed since the introduction of pertussis vaccine. Formerly, the prevalent serotypes were 1.2.3 and 1.2, whereas recently nearly all of the cultures isolated are serotype 1.3. British vaccines, he says, have not reflected this change.

Although the association of serotype and protection has not been confirmed by other workers (1, 3, 4, 6), it is of interest to know the serotypes of *B. pertussis* causing whooping cough in this country. In 1966 and 1967, under a contract with the National Institutes of Health, we produced antisera specific for the various *B. pertussis* factors, and serotyped certain cultures including those recently isolated from patients in various parts of the country. This report concerns the *B. pertussis* serotypes found during these years. Also included is a summary of the serotypes of some of the cultures isolated in the Grand Rapids laboratory from 1938 to 1968.

MATERIALS AND METHODS

Media. Cultures were maintained on Bordet-Gengou medium containing 20% sheep blood. Antigens for serotyping were prepared from growth on this solid medium.

Antisera. The antisera were produced by methods described in an earlier report (5). Antisera were adsorbed to specificity for each of the following factors: 1, 2, 3, 4, 5, and 6.

Serotyping procedure. The cultures collected for study during the period of the contract, isolated in 1966 and 1967, were serotyped with the specific antisera referred to above, using both slide and tube techniques. For later cultures, isolated in 1968, serotyping was done by the slide method only. Some of the cultures isolated before 1955 and maintained in the freeze-dried state, and which had been serotyped as part of the study reported in 1957 (5), were not retested now.

For the tube test, the rapid agglutination procedure described by Kendrick (7) was used. A series of appropriate serum dilutions, usually ranging from 1:10 to 1:2,500, was prepared, and 0.1 ml amounts were transferred to tubes (10 by 75 mm). The antigen, a suspension standardized to 20 opacity units, was added, 0.1 ml per tube. The tubes were shaken for 3 min at 75 rev/min in a mechanical shaker with a 4-inch (10.2 cm) stroke. The tests were incubated for 1 hr in a water bath at 37 C, followed by the addition of 0.5 ml of salt solution to each tube to facilitate reading. The agglutination results were read against a light with the aid of a hand lens and were recorded as negative, 1, 2, 3, or 4 plus for each tube. The titer was taken as the highest dilution in which a 2 plus reaction occurred.

For the slide test, approximately 0.025 ml of diluted

antiserum was placed toward one end of an ordinary microscope slide [1 by 3 inches (2.54 by 7.62 cm)], and a similar amount of saline was placed toward the other end. With a stiff bent needle or loop, a portion of a 24- to 48-hr culture of *B. pertussis*, grown on Bordet-Gengou medium (or other favorable solid medium), was removed and mixed with the drop of saline. The loop was flamed, and a similar portion of culture was mixed with the serum. The slide was rocked gently and observed for clumping. Specific agglutination occurred within 30 sec and was considered significant only if the control was negative.

Included with each set of tests were type cultures known to agglutinate with the specific antisera.

Cultures. To obtain recently isolated cultures of *B. pertussis* from as many sections of the country as possible, a request was published in the ASM News and letters were written to public health and hospital laboratories. The response was poor, suggesting that very few laboratories attempt to isolate *B. pertussis* from suspected whooping cough patients. The largest number of cultures from one laboratory was a group of 53 from Tulane University, where a study on drug susceptibility of *B. pertussis* was in progress. Cook County Hospital Laboratory, Chicago, Ill., sent 29 cultures isolated as part of their diagnostic service. The other sources were as follows: Ontario Provincial Laboratory, Windsor, Canada, 11 cultures; Emory University, Atlanta, Ga., 9 cultures; Children's Hospital, Los Angeles, Calif., 8 cultures; Cleveland Metropolitan General Hospital, Cleveland, Ohio, 3 cultures; Maryland State Laboratory, Baltimore, 1 culture.

These 114 plus 63 isolations in Grand Rapids brought the total to 177. Wide areas of the United States are not represented, notably the New England and Eastern states.

RESULTS

Cultures from different geographic areas. The serotypes of the 177 cultures collected from various parts of the country during 1966 and 1967 are shown in the Table 1. There are eight different combinations of antigens 1, 2, 3, 4, 5, and 6. The currently prevailing 1.3.6 pattern was shown by 148, or 83.6%. The next most common combination was 1.2.3.4.6, with 15 cultures or 8.5%; 10 of these were from New Orleans. There were 11 cultures which lacked factor 3; 2 of these were serotype 1.6, 3 were 1.2.4, 1 was 1.2.4.6, and 5 were agglutinated only by the factor 1 antiserum. With respect to factors 4, 5, and 6, it should be noted that 4 was always found in association with 2, 5 was identified in only one culture, and, with the exception of five cultures, 6 and 3 were usually found together.

To simplify the analysis and to make the results comparable with those of other workers, Table 2 lists the cultures according to the patterns 1.2.3, 1.2, 1.3, and 1 only. The proportion of 1.2.3 cultures from Louisiana, 19.0%, was greater than from other areas, and the proportion of 1.3 cultures was correspondingly smaller (68.0%). The three states supplying the largest numbers of cultures were Michigan (63), Louisiana (53), and Illinois (29). Serotype 1.3 cultures comprised 93.7% of the total in Michigan, 67.9% in Louisiana, and 86.2% in Illinois. Ten of the Louisiana cultures and four of those from Illinois were serotype 1.2.3. There were no 1.2.3 cultures from Michigan.

Besides the 177 *B. pertussis* cultures, 14 cultures of *B. parapertussis* were studied during the 2-year period. The sources were as follows: Louisiana, 4; Ohio, 2; California, 1; and Michigan, 7. *B. bronchiseptica*, the third species of the genus *Bordetella*, was represented by one culture sent from Boston. This was isolated from an 11-month-old child with symptoms of whooping cough.

Grand Rapids cultures, 1938 through 1968. To determine whether a change had occurred in the

TABLE 1. *Serotypes of B. pertussis cultures from different regions of the United States and Canada isolated during 1966 and 1967*

State or province	No. of cultures	Serotype							
		1.3.6	1.3	1.6	1.2.4	1.2.3.4.6	1.2.4.6	1.3.5.6	1 only
California	8	8							
Georgia	9	7				1			1
Illinois	29	24	1			4			
Louisiana	53	35	1	1	2	10	1		3
Maryland	1	1							
Michigan	63	59		1	1			1	1
Ohio	3	3							
Ontario	11	11							
Totals	177	148	2	2	3	15	1	1	5

TABLE 2. *Serotypes of B. pertussis cultures from different areas, according to presence of factors 1, 2, and 3*

State or province	No. of cultures	Serotype			
		1.2.3	1.2	1.3	1 only
California	8			8	
Georgia	9	1		7	1
Illinois	29	4		25	
Louisiana	53	10	3	36	4
Maryland	1			1	
Michigan	63		1	60	2
Ohio	3			3	
Ontario	11			11	
Totals	177	15	4	151	7
Per cent	100	8.5	2.3	85.3	4.0

TABLE 3. *Pertussis serotypes in Michigan from 1938 through 1968*

Period	No. of cultures	Serotype			Untypable[a]
		2.3	2	3	
1938–1940	9	5	2	0	2
1941–1945	37	16	1	13	7
1946–1950	40	20	3	16	1
1951–1955	74	14	2	57	1
1956–1960	83	5	2	76	0
1961–1965	36	1	0	33	2
1966–1968	97	0	2	93	2
Totals	376	61	12	288	15

[a] Includes those with factor 1 only.

B. pertussis serotypes in the Grand Rapids area, as observed in Britain, some of our stored lyophilized cultures were reconstituted and studied. The serotypes of 376 cultures isolated during the years 1938 through 1968 are given in Table 3. For convenience in comparing our results with those of Preston (10) and others, only factors 2 and 3 are considered. Since factor 1 serum was not available for some of the earlier tests, those cultures listed as untypable may have been agglutinable with factor 1 antiserum, although they were not reactive with either factor 2 or factor 3 serum. Of nine cultures isolated during the 3-year period of 1938 through 1940, five were serotype 2.3, two were serotype 2, two were untypable, and there was none in the serotype 3 group. For the 97 cultures listed for the 3 years 1966 through 1968, there were no 2.3 cultures. There were only 2 serotype 2 and 2 untypable cultures, and the remaining 93 were serotype 3.

During the five intervening periods, all comprising 5 years, there was a gradual shift from a predominance of serotype 2.3 to serotype 3 cultures. Actually, the greatest change occurred during the year 1953. At no time were many cultures found which possessed only antigen 2. In other words, factor 3 was present in nearly all cultures during the entire 30-year period, but at first it was in combination with factor 2, whereas recently factor 2 has almost disappeared, and the factor 3 cultures are almost the only ones found.

DISCUSSION

The 177 *B. pertussis* cultures collected from seven states and one Canadian province can scarcely be considered representative. It is disappointing that so few cultures were available for serotyping, and one must assume that very few laboratories offer diagnostic service for whooping cough. Among the cultures tested, serotype 1.3.6 predominated, and this was true for all of the geographic regions represented. Cultures with factor 2 were rare.

The five cultures which appeared to be serotype 1, and therefore like Preston's rare factor 1 culture designated 353Z, deserve some comment. *B. pertussis* cultures vary greatly in agglutinability; even smooth cultures which protect mice, stimulate the production of agglutinins in rabbits, and adsorb agglutinins may be inagglutinable or poorly agglutinable. To determine the serotype of such cultures, one must apply all three criteria, i.e., agglutinability, production of agglutinins, and ability to adsorb agglutinins. Obviously, these procedures are too cumbersome to be used frequently and were not applied with these cultures.

It is of interest to compare our results with those obtained in other countries. Chalvardjian (2) reported in 1965 that 56 of 58 *B. pertussis* cultures isolated in Toronto were of the 1.3 serotype and lacked factor 2.

Mebel (8) reported the serotypes of 431 *B. pertussis* cultures isolated in East Berlin during the years 1961 through 1967. Serotype 1.2.3 comprised 64.5% of the cultures and, since 1965, has predominated. Serotype 1.2 cultures accounted for 40.4% of the total. The 1.3 cultures which prevail in the United States were not found at all until 1964, and only 17 such cultures occurred in the entire study.

Joó (*personal communication*) serotyped 235 *B. pertussis* cultures isolated in Hungary during the years 1954 through 1957. He found serotypes 1.2.3 (or 1.2.3.4) 52 times (26%), serotype 1.2 (or 1.2.4) 79 times (40%), and serotype 1.3 (or 3) 70 times (33%). He also reported that, of 39 cultures isolated in 1966, 24 were serotype 1.3, 2 were

1.2.3, and 13 were untypable. In other words, the 1.3 cultures were the prevalent ones in 1966, and the change occurred between 1957 and 1966.

Although we need to learn more concerning the significance of *B. pertussis* serotypes, it seems worthwhile now to apply the method more widely to recently isolated cultures in order to recognize emerging epidemiological patterns.

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