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Ongoing Group B *Neisseria meningitidis* Epidemic in São Paulo, Brazil, Due to Increased Prevalence of a Single Clone of the ET-5 Complex

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Beginning in 1988, the incidence of meningococcal disease in the area of greater São Paulo began to surpass the upper confidence limit of an 8-year average incidence (from 1979 to 1986), thus characterizing a new epidemic in the region of greater São Paulo. This epidemic, which extended to 1990, was different from previous epidemics in that it was caused by serogroup B. The increased incidence of meningococcal disease was paralleled by an increased prevalence of a single group B clone, B:4:P1.15, of the ET-5 complex. ET-5 strains have been present in the greater São Paulo area since 1979; however, they have been associated with a high percentage of the group B disease only from 1987 to the present. On the basis of the increased incidence of group B disease in São Paulo, a mass vaccination program with a serotype 4:P1.15 meningococcal protein vaccine was undertaken. The impact of this vaccination program is under analysis.

The occurrence of meningococcal disease in Brazil has been monitored on a national level since the group A and C epidemics that occurred between 1972 and 1974 and reached levels in excess of 179 cases per 100,000 inhabitants of greater São Paulo in 1974. In April 1975, 95% of the population of greater São Paulo were vaccinated with a group A and C bivalent meningococcal polysaccharide vaccine. From 1980 onward, the annual incidence remained between 1.0 and 1.4 cases per 100,000 inhabitants. In recent years, the aspect of meningococcal disease having the greatest epidemiological relevance was the growing predominance of *Neisseria meningitidis* serogroup B, rather than the A and C serogroups, which had been responsible for the epidemics in Brazilian states in the past (10). The proportion of group B *N. meningitidis* among isolates that were serogrouped remained over 50% during most of the 1980s and now surpasses 70%.

In the city of São Paulo, meningococcal disease was clinically and epidemiologically diagnosed at the beginning of this century. From 1930 to 1945, the disease presented incidences between 2.0 and 4.0 cases per 100,000 inhabitants (9, 12). In 1945, it reached epidemic proportions which lasted until 1951, with a peak incidence in 1947 of 25 cases per 100,000 inhabitants. The *N. meningitidis* serogroup responsible for this epidemic was not determined.

After 1952, meningococcal disease lost its epidemic character. However, in 1971, a new and significant increase in the incidence of meningococcal disease was detected in the greater São Paulo area, and a new epidemic, which was caused by group C, began the following year. A peculiar phenomenon occurred during this period (from 1972 to 1974), in which there was an overlapping of two epidemic waves: one wave was caused by group C, and another, larger wave was caused by group A.

Beginning in 1988, the incidence of meningococcal disease in the greater São Paulo region again began to surpass the upper confidence limit of the 8-year average incidence (1979 to 1986), thus characterizing a new epidemic in the region as of 1988. This epidemic, which extended until 1990, was different from previous epidemics in that it was caused by serogroup B.

Since the group B capsular polysaccharide is poorly immunogenic, experimental outer membrane protein vaccines have been developed. However, knowing which serotypes to include in such vaccines has been a major problem, and this determination should be based on current epidemiological data. Serotypes within meningococcal serogroups are determined by the antigenic specificity of either the class 2 or the class 3 major outer membrane protein (6). All meningococcal strains have either a class 2 or a class 3 protein, but not both. Strains are also distinguished by subtype, which is based on their class 1 outer membrane protein (6). The combination of group, serotype, and subtype designations (for example, B:4:P1.15) can help distinguish among different epidemiologically related strains or clones.

A more recent technique for epidemiological tracing is multilocus enzyme electrophoresis typing, or electrophoretic type (ET) typing. ET typing examines electrophoretic differences in 10 to 15 enzymes required for growth of the bacteria. Both serotyping and ET typing identify strains or clones within a given serogroup; the important difference between them is that serotyping examines the antigenic diversity of important cell surface structures that may be involved in the induction of protective immunity.

The purpose of the present study was to document the dynamic clonal changes that may occur within a single serogroup over time. Meningococcal strains isolated in the

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TABLE 1. Distribution (by year) of *N. meningitidis* serogroup B strains in greater São Paulo

Yr	No. of isolates received	Strains serotyped	
		No.	% of total (for yr)
1977	103	14	14
1978	130	33	25
1979	97	23	24
1980	70	31	44
1981	82	29	35
1982	52	31	60
1983	78	39	50
1984	72	30	42
1985	101	29	29
1986	121	39	32
1987	123	36	29
1988	213	157	73
1989	218	120	55
1990	188	184	98
Total	1,648	795	48

greater São Paulo area from 1977 through 1991 were examined to determine the changes in the distribution of different serotypes and subtypes and to identify those responsible for the increase in meningococcal disease during the last 3 years.

MATERIALS AND METHODS

Origin of strains. Greater São Paulo includes the city of São Paulo (capital of the state of São Paulo) and 36 other nearby municipalities and had an estimated population of 16,450,000 in 1988. The majority of patients with meningococcal disease in this region are admitted to a single public hospital, Emilio Ribas Hospital, which specializes in contagious infectious diseases. The hospital is located in the city of São Paulo and uses the nearby Adolfo Lutz Institute as its diagnostic laboratory for cultures, spinal fluid analysis, and hematology.

All strains examined were from the strain collection of the Adolfo Lutz Institute. The percentage of *N. meningitidis* group B strains selected to be serotyped for each year depended on the number of strains lyophilized and strain viability (Table 1).

Grouping and serotyping. Following biochemical identification, the strains were serogrouped by the slide agglutination technique, in which antisera against the nine major capsular serogroups of *N. meningitidis* were used. These antisera were prepared by the National Reference Center for Meningitis at the Adolfo Lutz Institute. The 795 selected serogroup B strains were serotyped and subtyped by a dot immunoblot technique (7) with monoclonal antibodies and outer membrane vesicles. Monoclonal antibodies for serotypes 2a (1673F2), 2b (1082E7), 4 (2303C5), 8 (2725H6), and 15 (1951C8) and subtypes P1.2 (1649C7) and P1.15 (2731C6) were from the Center for Biologics Evaluation and Research, Bethesda, Md.; others were kindly provided by Wendell D. Zollinger of the Walter Reed Army Institute of Research, Washington, D.C.

Outer membrane vesicles. Outer membrane protein preparations used for serotype and subtype analysis were prepared by a rapid extraction procedure. Each strain was grown overnight on one tryptic soy agar plate (Difco, Detroit, Mich.) containing 1% normal horse serum. Cells were harvested from the plate and suspended in 1.5 ml of extraction fluid (0.2 M LiCl, 0.1 M sodium acetate [pH 6.0]). Glass beads were added, and the outer membrane vesicles were extracted by vigorous shaking at 50°C for 2 h. Cells were removed by centrifugation at 10,000 × *g* for 20 min. The outer membrane vesicles were pelleted from the cell supernatant by ultracentrifugation at 100,000 × *g* for 2 h and then were used in the dot blot immunoassay.

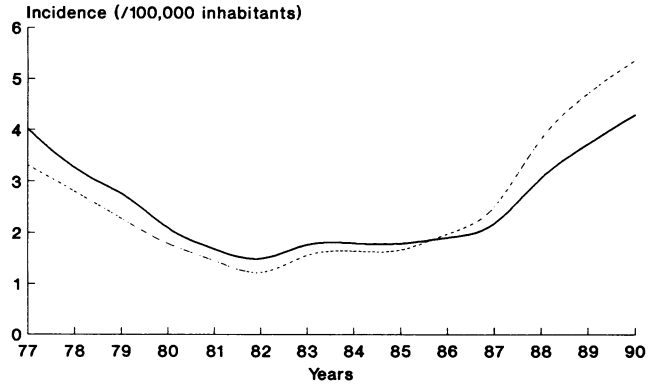

FIG. 1. Incidence of *N. meningitidis* disease in São Paulo State (solid line) and greater São Paulo (broken line) from 1977 to 1990.

ET typing. Methods for protein extract preparation, starch gel electrophoresis, and enzyme detection have been described previously (13). Each isolate was characterized by its combination of alleles for the 13 enzymes assayed and its distinctive multilocus genotypes (also designated ETs), and each isolate was compared with those previously identified (4).

Source of epidemiological data. The data pertaining to meningococcal disease among people residing in the metropolitan region of São Paulo are the product of the System for Epidemiological Vigilance for the state of São Paulo and were consolidated and analyzed by the Center for Epidemiological Vigilance.

RESULTS

The incidence of meningococcal disease, including meningitis and septicemia, in greater São Paulo was 3.31 cases per 100,000 inhabitants in 1977 and fell to a low of 1.05 cases per 100,000 inhabitants in 1982 (Fig. 1). The incidence has increased steadily since 1986 and reached 5.39 cases per 100,000 inhabitants in 1990.

The incidence (by month) of meningococcal disease in the greater São Paulo region is shown in Fig. 2. The incidence began to surpass the upper confidence limit of the 8-year average incidence (from 1979 to 1986) in some months of 1987, but from 1988 onward, an epidemic became evident. Note the seasonality in the incidence of disease.

The serogroup distribution over the last 17 years has been analyzed for the greater São Paulo region (Fig. 3). The proportion of meningococcal disease caused by group B has been about 80% of all meningococcal disease since 1983. However, in 1989, the number of cases of group C disease increased, and it doubled again in 1990. Serogroup A strains have not been isolated since 1982. In 1986, there was one counterimmunoelectrophoresis-positive case of group A disease, but it was not confirmed by culture. Figure 3 also shows the percentage of meningococcal disease among all culture- and antigen-diagnosed cases of bacterial meningitis.

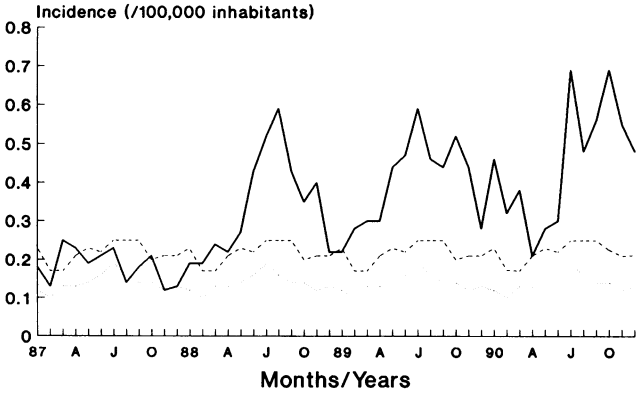

FIG. 2. Coefficient of incidence (by month) of *N. meningitidis* disease in greater São Paulo from 1987 to 1990 (——). A, April; J, July; O, October. Data from 1979 to 1986 were used to establish the mean (·····) and upper limit (– – –) of the endemic levels.

Along with the increased proportion of meningococcal disease due to serogroup B, there have been major changes during the study period in the strains causing disease. Figure 4 shows the number of strains of the predominant group B serotypes found in the São Paulo area since 1977. Serotype B:4 has been prevalent since 1984 and has increased in prevalence each year through 1989, when it accounted for 70% of all group B isolates. Until 1982, nonserotypeable (NT) strains accounted for the highest percentage of isolates among serogroup B. B:NT strains have now been replaced by serotype 4, and B:4:P1.15 strains have accounted for over 50% of all B isolates since 1988.

Although serotype 2b has been reported elsewhere, it was infrequently isolated in the greater São Paulo area. In 1988, serotype 2b accounted for only 1.27% of group B isolates but accounted for 2.7% in 1990. On the other hand, serotype B:2a made up about 4.37% of the serotypes isolated between 1977 and 1984, but no further isolates were recovered until 1990 (one isolate).

The distributions of serotype B:4:P1.15 and of the ET-5 complex are shown in Fig. 4. Strains belonging to the ET-5 complex have increased in parallel with the incidence of B:4:P1.15 over the last several years. Nearly all of the B:4:P1.15 isolates belonged to the ET-5 complex.

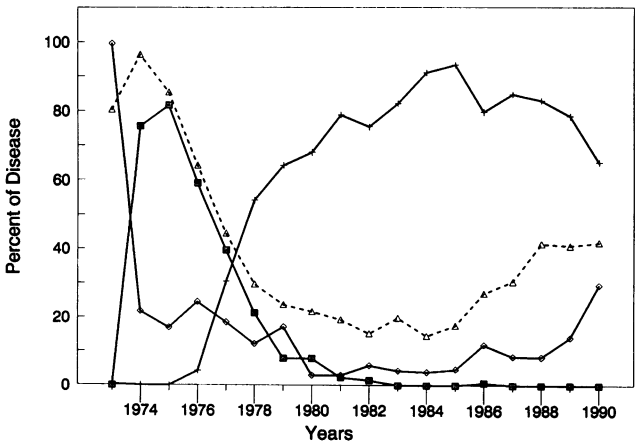

FIG. 3. Distribution of serogroups among disease isolates of *N. meningitidis* recovered between 1973 and 1990 from greater São Paulo. The percentage of total culture- or antigen-diagnosed bacterial meningitidis due to *N. meningitidis* is also shown. □, group A; +, group B; ◇, group C; △, bacterial meningitis.

DISCUSSION

Group B meningococcal strains that cause disease in different countries have been shown to be very heterogeneous. Within recent years, outbreaks and epidemics have been caused by serotypes 2a, 2b, 4, 8, and 15 (5). Prior to the mid-1970s, much of group B disease in several parts of the world was caused by serotype 2a (1, 5). During the late 1970s, a shift from serotype B:2a to B:2b was seen in the United States (5) and Canada (3). Similar shifts in serotype 2 have occurred in England, Wales, Norway, Denmark, the Netherlands, Iceland, and the Faroe Islands (1, 8, 11). Serotype 15 has been hyperendemic in Norway since 1974. More recently, B:15:P1.16 strains have caused outbreaks of meningitis in Great Britain, Iceland, Denmark, the Faroe Islands, and the Netherlands (8, 11). Clones representing the responsible serotype 15 strains have been classified by ET typing into a number of closely related ETs designated the ET-5 complex, which is characterized by genetic homogeneity and an ability to cause serious epidemics (4). The ET-5 complex seems to have spread widely in many countries during the late 1970s and 1980s (4).

The Emilio Ribas Hospital cares for nearly 80% of patients with meningococcal disease in greater São Paulo. Therefore, this hospital is a true reflection of greater São Paulo in terms of meningococcal disease. The incidence for greater São Paulo was found to approximately parallel that of the state of São Paulo, but in 1986, greater São Paulo began to present higher coefficients of incidence in relation to those of the state (Fig. 1). Between 1980 and 1986, the incidence remained approximately constant, varying between 1.0 and 2.0 cases per 100,000 inhabitants, but increased thereafter, reaching coefficients higher than in 1977. In 1990, the incidence was 5.39 cases per 100,000 inhabitants. There was also a great change in relative prevalence of the serogroups during this period (from 1977 to 1990). The percentage of disease due to serogroup A fell from 39.64% in 1977 to 1.45% in 1982, while the percentage of disease due to serogroup B increased from 30.47 to 75.35% in the same period. The percentage of meningococcal disease due to serogroup B continued to rise until 1985, when serogroup B disease represented 93.52% of all the cases of meningococcal disease, but has decreased since. It is interesting that during this period, there was also an increase in meningococcal disease, from 26.96% in 1986 to 41.78% in 1990, in relation to total bacterial meningitis (Fig. 3).

By February of 1988, the incidence of meningococcal disease began to persist above the upper confidence limit of the 8-year average incidence, which denoted the start of an epidemic; incidence reached 4.06 cases per 100,000 inhabitants in 1988. Even higher levels were seen in 1989 and 1990 (4.74 and 5.39 cases per 100,000 inhabitants, respectively).

Regarding the serotypes of serogroup B, there was an inverse relationship between serotype B:4 and nontypeable strains during the period analyzed (Fig. 4). The increased incidence of meningococcal disease was paralleled by an increased prevalence of a single group B clone, B:4:P1.15, of the ET-5 complex. The percentage of B:4 strains remained approximately constant at around 25% between 1977 and 1982. From 1982 to 1990, the increase in the percentage of serogroup B was accompanied by an increase in the incidence of serotype B:4 (from 42% in 1982 to 80% in 1990) and

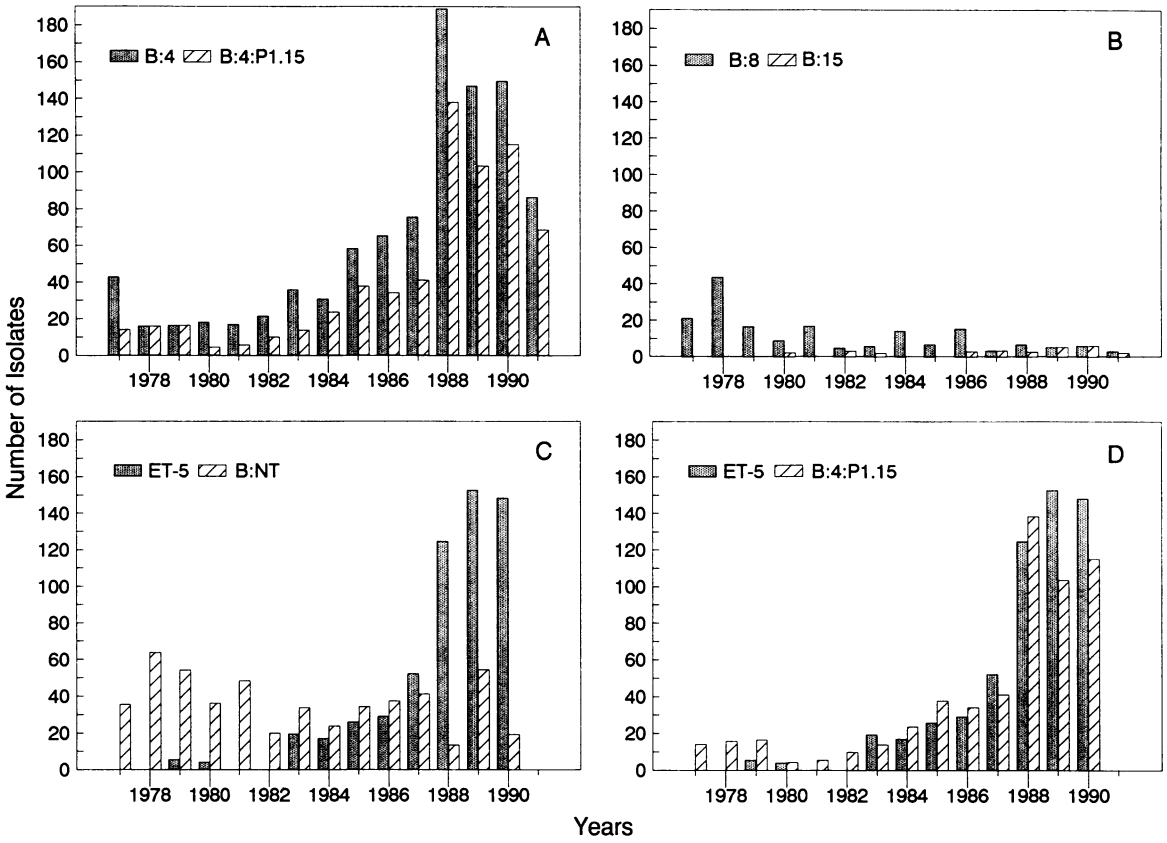

FIG. 4. Percentage of different *N. meningitidis* group B serotypes isolated in greater São Paulo between 1977 and 1991. The relationship of serotype 4 strains to the appearance of the ET-5 complex is shown (C and D). ET-5 complex data from 1991 were not available. To adjust for the variable percentage of meningococcal isolates serotyped for the years 1977 to 1990 (90% were serotyped in 1991), the numbers of strains were normalized on the basis of the percentage of strains examined (Table 1).

a drop in B:8 and nonserotypeable strains. However, from 1986 to 1990, we had an increase in the percentage of strains of serotype B:4, which was due only to an increase in the incidence in B:4:P1.15, since the percentage of B:4:nst strains remained about the same.

Group B isolates recovered in 1981 from Cuban immigrants to Miami, Fla., were B:4:P1.15 (4). The B:4:P1.15 strains from Miami, Cuba, and greater São Paulo were found to be the clone that represents the ET-5 complex (4). ET-5 strains have been present in the greater São Paulo area since 1979; however, they have been associated with a high percentage of the group B disease only from 1987 to the present. Since the B:4:P1.15 strain was responsible for 61% of all B strains for 1990 examined in this study, it appears that the Cuban clone has replaced other group B strains such B:4:nst and various B:NT strains in the São Paulo area.

Serotype 4 has now become a dominant group B serotype in many countries. This trend appears to be worldwide, with Abdillahi and Poolman (2) reporting that 36% of the group B isolates from 20 countries are serotype 4 and that another 14, 8, and 25% are serotypes 15 and 2 and nontypeable, respectively.

While ETs are derived by examining 10 to 15 genetic loci and can therefore define clones for epidemiological purposes, these loci are for housekeeping enzymes and have little to do with host immunity. It is therefore important to examine *N. meningitidis* isolates for both ETs and the antigenic specificities of the cell surface major outer membrane protein serotypes and subtypes.

On the basis of the demonstrated high prevalence of serotype 4 strains in the greater São Paulo area, a mass vaccination campaign with a Cuban B:4:P1.15 outer membrane vaccine combined with the meningococcal group C polysaccharide was carried out in 1990. The impact of this vaccination program is currently under analysis at the Adolfo Lutz Institute and Center for Epidemiological Vigilance.

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