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Seroprevalence of Immunoglobulin M (IgM) and IgG Antibodies to Polysaccharides of *Streptococcus pneumoniae* in Different Age Groups of Ecuadorian and German Children

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The age-specific prevalence of serum immunoglobulin M (IgM) antibody to capsular polysaccharides of *Streptococcus pneumoniae*, as detected by enzyme-linked immunosorbent assay, was studied in 1,301 Ecuadorian children enrolled in a national nutrition and health survey. This prevalence was 6% in infants <6 months old and increased to 28% in children 6 to 11 months old, 49% in those 12 to 17 months old, and 58% in those 18 to 23 months old. About 80% of the 5-year-old children had this antibody. When tested separately against six different capsular polysaccharides, serum IgM antibody reacted with decreasing frequency with serotype 3, 8, 19, 6, 23, and 1 capsular polysaccharides. We did not observe a broadening of the antibody response with increasing age in the sense that more and more serotypes were recognized. A similar age-related prevalence was found for IgM antibody to the species-specific C-polysaccharide of *S. pneumoniae* and for IgG antibody to capsular polysaccharides of *S. pneumoniae*. A smaller German serum collection showed a comparable age-related prevalence of pneumococcus-specific serum IgG and IgM antibodies. The highest incidence of respiratory diseases was observed in 1- and 2-year-old Ecuadorian children. It thus seems that acquisition of serum antibody to *S. pneumoniae* reflects more the developmental maturation of an immune response than an actual exposure to different pneumococcal serotypes.

Diarrhea and respiratory infections are the principal causes of death in the world (27). Of the 15 million children <5 years old who die each year (14), 96% die in developing countries. About 30% of these children die from pneumonia (28). It has been clearly established that the main cause of death in young children in developing countries is bacterial pneumonia, whether primary or secondary to viral infections, and that the predominant organisms responsible are *Streptococcus pneumoniae* and *Haemophilus influenzae* (24). Several trials of the efficacy of pneumococcal capsular polysaccharide vaccines in preventing pneumococcal disease in young children have been undertaken (1, 21, 22). A common finding in all these studies was that only a transient protective effect, if any, could be demonstrated in children <2 years old.

It is known that vaccination with purified pneumococcal polysaccharides induces only a poor antibody response in children <5 years old (8, 18). It is less well known whether young children respond as poorly to natural *S. pneumoniae* exposure (12, 13, 26). Therefore, we analyzed the age-related prevalence of serum immunoglobulin M (IgM) and IgG antibodies to pneumococcal polysaccharides in 1,480 children whom we had previously characterized for antibody responses to a number of viral, bacterial, and food antigens (3–7).

MATERIALS AND METHODS

Respiratory disease. Information about the occurrence of an episode of respiratory disease in the 15 days preceding data and sample collection was obtained from the parent. Acute respiratory disease was defined as an inflammation of the nose or the throat with a discharge combined with fever. A significant respiratory disease was defined as breathlessness in addition to the other symptoms.

Enzyme-linked immunosorbent assay (ELISA). Purified pneumococcal polysaccharides of the following *S. pneumoniae* types, identified by their U.S. (Danish) designations, were obtained from the American Type Culture Collection (Rockville, Md.): 1 (1), 2 (2), 3 (3), 4 (4), 6 (6A), 8 (8), 9 (9N), 12 (12F), 19 (19F), 23 (23F), 51 (7F), and 56 (18C). The antigens were prepared by Merck Sharp & Dohme during the manufacture of the pneumococcal vaccine and then repackaged by the American Type Culture Collection for distribution as research reagents.

In type-specific ELISA, it is very important to block the nonspecific binding of antibodies to the pneumococcal group antigen C-polysaccharide (25). Therefore, purified C-polysaccharide was purchased from the Statens Seruminstitut (Copenhagen, Denmark).

The mixture of the 12 pneumococcal polysaccharides or, alternatively, only one capsular polysaccharide or only C-polysaccharide was added to carbonate-bicarbonate buffer (pH 9.6) at a concentration of 10 μg/ml, and microtiter plates (Dynatech) were coated with the polysaccharide preparations. The plates were incubated for 3 h at room temperature with shaking and for 2 days at 4°C and then stored frozen. Serum samples to be tested were diluted in phosphate-buffered saline–Tween 20 (0.05%) to a standard dilution of 1:100. Siber et al. (25) proposed that a standardized pneumococcal ELISA should include absorption of test sera and standards with C-polysaccharide. Therefore, sera were first incubated for 1 h at room temperature with C-polysaccharide (10 μg/ml) before they were added to the test plates when antibody against capsular polysaccharides was measured.

After overnight incubation of the sera on the test plates, bound antibodies were revealed with affinity-purified goat antibody to human IgM or IgG coupled to alkaline phosphatase (Sigma, St. Louis, Mo.). Siber et al. (25) indicated

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that it may not be routinely necessary to remove rheumatoid factor (RF) (IgM-class antibodies to IgG) from test specimens in order to quantitate IgM antibody to capsular polysaccharides. Therefore, no efforts to measure RF in Ecuadorian sera, which were available only in limited amounts, were made. However, 134 serum samples from German children were tested for RF with a commercial latex RF test (Rapi Tex RF; Behringwerke, Marburg, Germany); five RF-positive serum samples were identified. Three had IgG antibody to pneumococcal polysaccharides, but none scored positive in our IgM ELISA.

For the prevalence analysis, a serum sample was counted as positive if the absorbance on the test plate was at least twice that on the control plate and at least 0.1 optical density (OD) unit greater than the OD of the control plate coated with coating buffer alone. Standardization of the reagents with respect to sensitivity and specificity for the different immunoglobulin classes was done, according to the method of Yolken et al. (29), with plates to which chromatographically purified IgG, IgM, or IgA (Cappel Laboratories, West Chester, Pa.) had been added at various dilutions. Conjugate dilutions were chosen so that 30 ng of the specific immunoglobulin per ml coating microtiter plates still produced an absorbance of 0.1 OD unit. A concentration of the nonspecific human immunoglobulin 1,000-fold higher than that of the specific immunoglobulin was necessary to obtain a similar absorbance and thus demonstrated the specificity of the conjugates. The specificity and sensitivity of the conjugates were independently confirmed with commercial standard sera (Behringwerke) containing known concentrations of IgG and IgM antibodies. As we worked with a 1:100 serum dilution, an OD of 0.1 corresponded to 3 μg of capsular polysaccharide-specific antibody per ml of undiluted serum sample. Assuming that 1 μg of antibody protein corresponds to about 150 ng of antibody nitrogen (13), our cutoff corresponds to 450 ng of specific antibody nitrogen per ml of serum.

Sera from Ecuador. As described previously (9), 7,798 children were studied for a representative nutritional and health survey of the ~1.2 million children <6 years of age in Ecuador (total population, 9.6 million). A representative subset of 1,620 children was selected for serum collection. Of these, 540 children lived in two metropolitan areas (Quito and Guayaquil; populations, 1.1 million and 1.6 million, respectively), 540 children lived in 14 predominantly urban regions (cantons), and 540 children lived in 14 predominantly rural cantons. A total of 96% of the population of children in Ecuador was represented; children in the remote Galápagos Islands and the thinly inhabited Amazon Basin (El Oriente region; <4% of the total population) were excluded from the study for logistical reasons.

Serum samples were obtained from 1,570 children (97%). Samples for detection of pneumococcal antibodies were available from 1,301 children (80%). The sera were collected from March to November 1986 by finger prick for infants (age, <6 months) and by venipuncture (antecubital vein) for older children. Sera were stored frozen.

About 50 serum specimens were available from children in each 2-month age interval from age 0 to 5 years. The age distribution was homogeneous with respect to gender, month of serum collection, geographic region, population density, and socioeconomic level.

Sera from Germany. Serum samples from 179 infants and children, collected for diagnostic purposes unrelated to this study, were obtained from the University Children's Hospital at Bochum, Federal Republic of Germany. All specimens were obtained from children who were hospitalized during the years 1984 and 1985. Children hospitalized with hematologic disease requiring blood transfusion were excluded. All infants were residents of the Bochum area.

RESULTS

Prevalence of pneumococcal antibodies in German children. As indicated by ELISA, 88% of German mothers had serum IgM antibody to a mixture of 12 capsular polysaccharides of *S. pneumoniae*. The specificity of this antibody reactivity was demonstrated by the absence of this antibody from the cord sera of their respective infants.

Only 4% of German children younger than 16 months had IgM antibodies against pneumococcal polysaccharides; this prevalence increased to 20% in 16- to 24-month-old children and reached a 50% prevalence level in children 2 to 4 years old (Fig. 1).

The serum collection was retested for IgM antibody to the C-polysaccharide (16), which is common to all pneumococci (20). This was done to exclude the possibility that we missed antibodies to capsular polysaccharides of serotypes not included in the ELISA. This was apparently not the case: in German children the prevalence of antibody to C-polysaccharide was not higher than the prevalence of antibody to the capsular polysaccharides (Fig. 1). Totals of 95% of German mothers and 87% of the cord sera had IgG antibody to capsular polysaccharides of *S. pneumoniae*. As anticipated for maternally derived antibodies, this prevalence decreased over the next months of life to reach a minimum of 7% in 12- to 16-month-old children (Fig. 1). For German children between 16 and 48 months of age, a prevalence increase of specific IgG antibody that paralleled the prevalence increase seen for specific IgM antibody was observed.

Prevalence of pneumococcal antibodies in Ecuadorian children. A total of 6% of the Ecuadorian infants <6 months old had serum IgM antibody to the mixture of capsular polysaccharides, as indicated by ELISA (Fig. 2). This prevalence increased to 28% in the second 6 months of life and reached 49% in 1- to 1½-year-old children and 58% in 1½- to 2-year-old children. Between 66 and 79% of the Ecuadorian children >2 and <6 years old had serum IgM antibody to capsular polysaccharides. A very similar age-related prevalence was found when serum IgM antibody to the common C-polysaccharide of *S. pneumoniae* was measured by ELISA (Fig. 2).

The prevalence of sera positive for both antibody specificities showed an age development parallel to that of the prevalence of sera positive for either antigen alone, although the doubly positive sera had a somewhat lower prevalence level (compare Fig. 2B with 2A). In addition, with the increasing age of the children we observed a steady prevalence decrease of sera negative for both antibody specificities (Fig. 2B). In the first 6 months of life, 83% of the sera from Ecuadorian children were negative for antibody to both antigens; this prevalence of doubly negative sera fell to 57% in the second 6 months and to 25% in the second year of life.

Because the definition of cutoff points for prevalence analysis is intrinsically arbitrary, we also studied mean net OD for the Ecuadorian serum samples by ELISA. Essentially, this analysis revealed the same age dependence of *S. pneumoniae*-specific IgM antibody as the prevalence analysis (compare Fig. 3 with 2A).

A total of 77% of the Ecuadorian children <2 months old

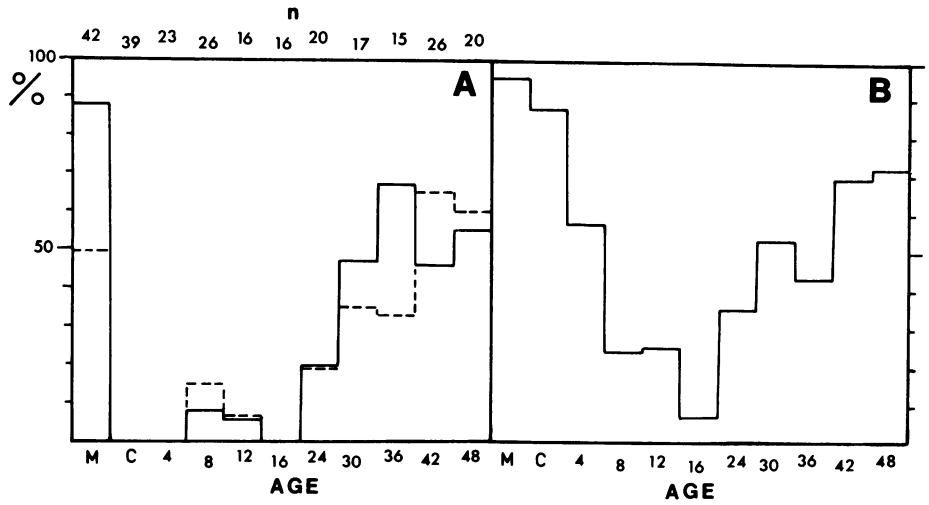

FIG. 1. Prevalence of serum IgM antibody to capsular polysaccharides (——) and C-polysaccharide (– – –) (A) and of serum IgG antibody to capsular polysaccharides (B) of *S. pneumoniae* in German children. n, number of children in each age group; AGE, upper age limit of each group (in months); M and C, matched maternal sera and cord sera from the same area.

had serum IgG antibody to the mixture of capsular polysaccharides, as indicated by ELISA (Fig. 4A). As anticipated for passively acquired maternal antibody, the prevalence of IgG antibody to pneumococci decreased to 22% in 2- to 4-month-old infants and remained low up to 8 months of age. A gradual prevalence increase from 29 to 75% was seen for IgG antibody in children between 8 and 20 months of age, and this prevalence remained high in older children. A very similar age development of pneumococcus-specific IgG was observed when we analyzed the mean net OD by ELISA

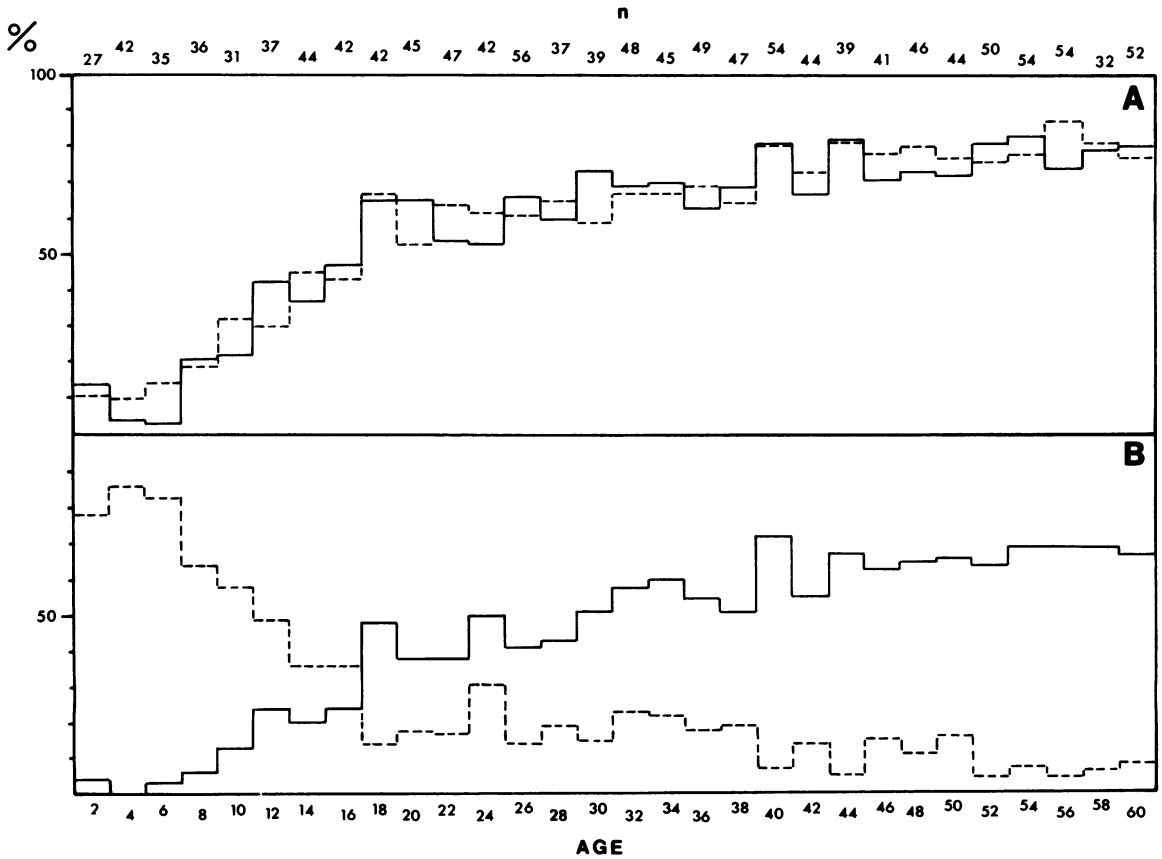

FIG. 2. (A) Prevalence of serum IgM antibody to capsular polysaccharides (——) and C-polysaccharide (– – –) of *S. pneumoniae* in Ecuadorian children. (B) Prevalence of sera positive (——) or negative (– – –) for IgM antibody both to capsular polysaccharides and to C-polysaccharide of *S. pneumoniae*. n, number of children in each 2-month age group; AGE, upper age limit of each group (in months).

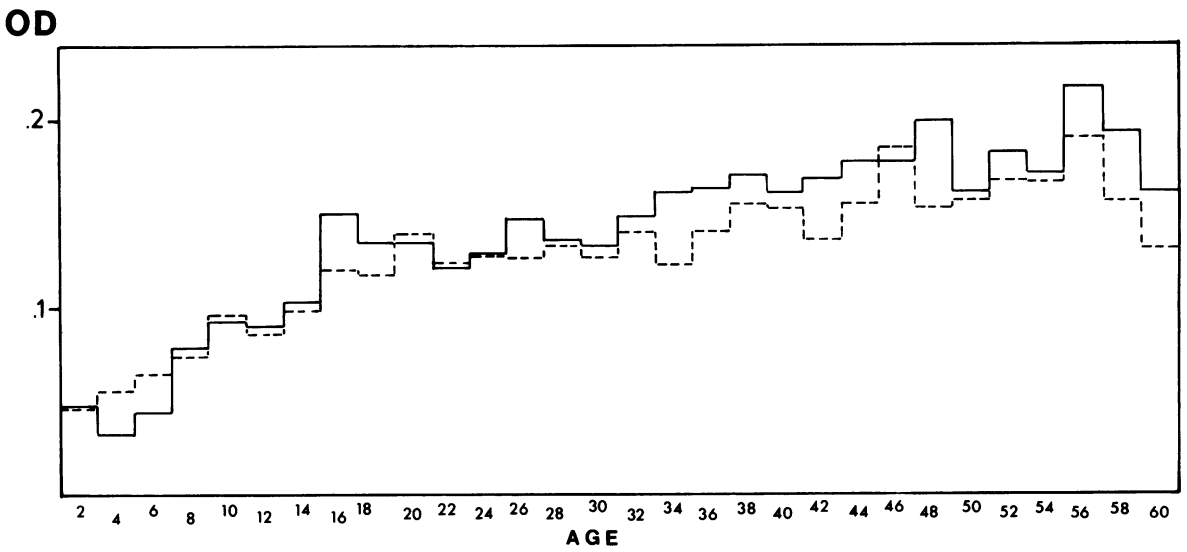

FIG. 3. Mean OD measured by ELISA for serum IgM antibody to capsular polysaccharides (——) and C-polysaccharide (- - -) of *S. pneumoniae* in 1,301 Ecuadorian children of the indicated ages (in months).

(Fig. 4B). Note also that in our seroprevalence study, pneumococcus-specific IgM antibodies did not appear at an earlier age than specific IgG antibodies.

Specificity of pneumococcal antibodies in Ecuadorian children. For each 1-year age group of the Ecuadorian children, 30 to 40 serum samples were randomly selected for further analysis. The only restriction was that they had to be positive for IgM antibody to the mixture of 12 capsular

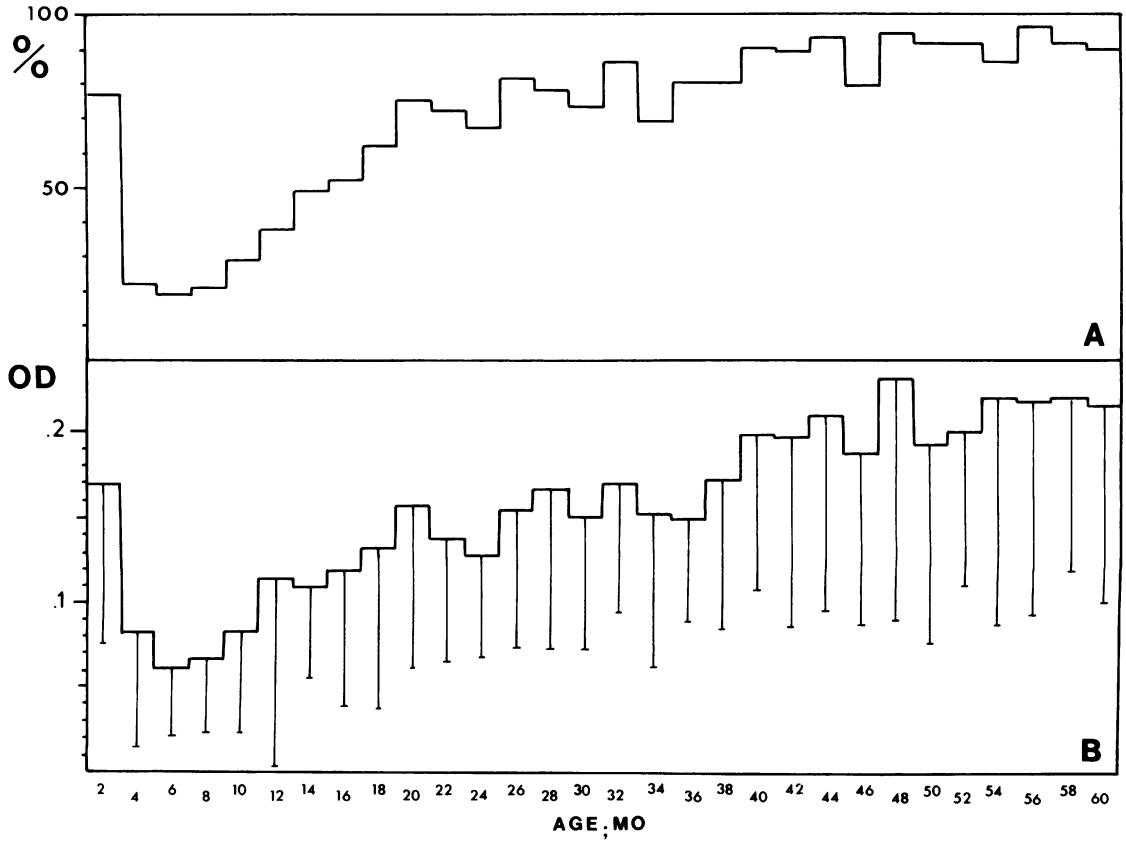

FIG. 4. (A) Prevalence of serum IgG antibody to capsular polysaccharides of *S. pneumoniae* in Ecuadorian children of the indicated ages. (B) Mean OD (±standard deviation) measured by ELISA for serum IgG antibody to capsular polysaccharides of *S. pneumoniae* in Ecuadorian children of the indicated ages.

TABLE 1. Prevalence of IgM antibody to capsular polysaccharides of *S. pneumoniae* serotypes in 188 Ecuadorian children, as indicated by ELISA

Age of children (yr)	No. of children	No. (%) of sera reacting with capsular polysaccharide of serotype:					
		1	3	6	8	19	23
1	32	5 (16)	13 (41)	7 (22)	11 (34)	9 (28)	6 (19)
2	36	5 (14)	25 (69)	14 (39)	23 (64)	19 (53)	9 (25)
3	33	1 (3)	27 (82)	9 (27)	19 (58)	8 (24)	4 (12)
4	44	5 (11)	37 (84)	20 (45)	28 (64)	22 (50)	6 (14)
5	43	2 (5)	34 (79)	18 (42)	27 (63)	15 (35)	10 (23)
Total	188	18 (10)	136 (72)	68 (36)	108 (57)	73 (39)	35 (19)

polysaccharides, as indicated by ELISA. These sera were then tested separately for IgM antibody to capsular polysaccharides of six serotypes of *S. pneumoniae* frequently associated with pneumococcal disease.

Of the 188 serum samples, 136 (72%) and 108 (57%) had IgM antibody reactive with type 3 and 8 capsular polysaccharides, respectively (Table 1). Totals of 36 and 39% of the sera reacted with type 6 and 19 capsular polysaccharides, respectively. Only 10 and 19% of the 188 serum samples reacted with type 1 and 23 polysaccharides, respectively. No age-related prevalence increase was seen for antibody to type 1, 6, 19, and 23 polysaccharides. Two-year-old children had a higher prevalence of antibody to type 3 and 8 polysaccharides than 1-year-old children (Table 1).

The prevalence of sera which failed to react with any of the six polysaccharide types decreased in the second year of life compared with the first year of life (Table 2). The 188 serum samples were then analyzed for reactivity with multiple types of the six capsular polysaccharides tested (Table 2). However, we did not observe an age-related increase in the prevalence of sera with multiple reactivities.

Age-related prevalence of acute respiratory illness in Ecuadorian children. About 25% of the 1- and 2-year-old Ecuadorian children had histories of a recent significant episode of respiratory illness. This prevalence decreased to about 20% in the third year of life and to less than 20% in the fourth and fifth years of life (Fig. 5).

When a less restrictive definition of respiratory disease, omitting breathlessness as an inclusion criterion, was used, about 38% of children 6 to 24 months old had histories of a recent episode of respiratory disease. This prevalence was somewhat lower in children <6 and >24 months of age (Fig. 5).

TABLE 2. Specificity of pneumococcal IgM antibodies in different age groups of Ecuadorian children, as indicated by ELISA

Age of children (yr)	No. of children	No. (%) of sera reacting with capsular polysaccharides of indicated no. of *S. pneumoniae* serotypes						
		0	1	2	3	4	5	6
1	32	15 (47)	7 (22)	2 (6)	2 (6)	1 (3)	2 (6)	3 (9)
2	36	8 (22)	6 (17)	5 (14)	2 (5)	9 (25)	2 (5)	4 (11)
3	33	5 (15)	8 (24)	9 (27)	5 (15)	4 (12)	1 (3)	1 (3)
4	44	2 (5)	12 (27)	8 (18)	6 (14)	10 (23)	4 (9)	2 (5)
5	43	7 (16)	6 (14)	14 (33)	2 (5)	6 (14)	7 (16)	1 (2)
Total	188	37 (20)	39 (21)	38 (20)	17 (9)	30 (16)	16 (8)	11 (6)

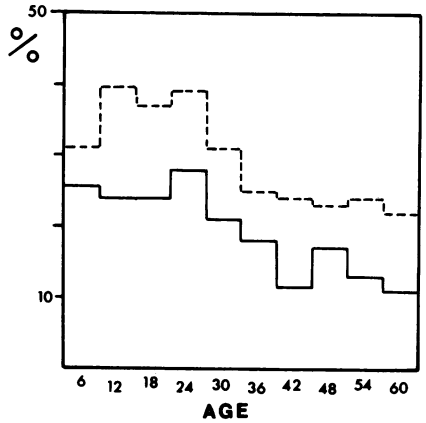

FIG. 5. Prevalence of a recent moderate (- - -) or significant (——) episode of undifferentiated respiratory disease in 1,301 Ecuadorian children according to age; AGE, upper age limit (in months) for each age group.

DISCUSSION

Clinical and experimental observations have firmly established the association between the presence of type-specific serum antibody and protection against infection by homologous pneumococcal organisms. However, there is limited information on what constitutes a minimal protective level of antibody for each pneumococcal serotype. Clinical and experimental data support the notion that a concentration of 250 to 300 ng of antibody nitrogen per ml constitutes the threshold of protection (17, 19). Our cutoff level for the seroprevalence study was set at 450 ng of antibody nitrogen per ml. Note, however, that we measured antibody to a mixture of 12 different capsular polysaccharides. When a subset of sera were tested separately for six individual capsular polysaccharides, only antibody to serotypes 3 and 8 crossed the protective threshold in the majority of the sera, whereas antibody to serotypes 1 and 23 mostly failed to attain the protective level. Thus, a child classified as seropositive in our seroprevalence analysis is likely to be protected against infection with serotype 3 and 8 pneumococci but also likely to be susceptible to infection with serotype 1 and 23 pneumococci.

In Ecuadorian children, respiratory diseases were more commonly observed than diarrheal diseases. In contrast to diarrheal diseases, for which the peak prevalences were noted in 12- to 24-month-old children (3), the peak prevalences of significant respiratory disease were observed in children younger than 12 months. In concurrence with these clinical observations, Ecuadorian children acquired serum IgM antibody to respiratory syncytial virus (7) at a much earlier age (more than 50% of 2- to 4-month-old infants had specific IgM antibody) than they acquired antibody to enteric pathogens (rotavirus [3] and enterotoxigenic *Escherichia coli* [4]). Acquisition of antibody to *S. pneumoniae* was, however, conspicuously delayed: 50% prevalence levels for serum IgG and IgM were reached only in Ecuadorian children older than 16 months. This was not due to a lack of sensitivity in our ELISA, as 88% of adult sera had such IgM antibody. Neither was it due to a lack of inclusion of appropriate antigens in ELISA, since the species-specific antigen of *S. pneumoniae*, C-polysaccharide, did not reveal higher antibody prevalences.

The interpretation that the majority of Ecuadorian children did not experience *S. pneumoniae* exposure before 16

months of age does not, however, concur with epidemiological data for *S. pneumoniae* infections. The mean age at acquisition of the first *S. pneumoniae* strain was 6 months in a prospective study with North American infants (11). Acquisition of a second and a third strain occurred, on average, 6 and 4 months after acquisition of the first and second strains, respectively. Early, intensive exposure to *S. pneumoniae* is also common for infants from tropical developing countries. A study of neonates in Papua New Guinea showed that 60% had acquired *S. pneumoniae* by the age of 15 days and all had acquired the organism by 3 months of age; the density of the colonization was high (10). The relative absence of a serological response to *S. pneumoniae* in young Ecuadorian children might thus indicate their inability to mount an antibody response to *S. pneumoniae*. There are substantial data in the literature to support this view. The immune response to pneumococcal vaccination was poor in children younger than 5 years of age (8, 18). In addition, during the first 2 years of life, only small and brief antibody responses to pneumococcal polysaccharides were observed (12, 13).

The analysis of the serotype specificity of the Ecuadorian sera provided some indirect evidence to support this view. The most prevalent antibody reactivity was against the adult serotypes 3 and 8 and not against the pediatric serotypes 6, 19, and 23. Note, however, that the distinction between adult and pediatric serotypes might be questionable for developing countries (23). Interestingly, vaccination trials in children also showed a pronounced increase in levels of antibody to serotypes 3 and 8 and only very weak increases in levels of antibody to serotypes 6, 19, and 23 (8, 18). It thus seems that the antibody specificity of the Ecuadorian sera reflects more the relative antigenicity of the different polysaccharide antigens than an actual exposure to different pneumococcal serotypes. Antibodies to serotypes 3 and 8 are known to cross-react, and it might be noted, since these children were previously studied for antibodies to *E. coli* (4), that the antibodies also cross-react with *E. coli* K7, 08, 09, and 013 polysaccharides (15).

The mixture of 12 types of capsular polysaccharides for the screening assay has the substantial drawback of making it impossible to determine whether the age-related prevalence increase is due to a broader response (antibodies to more types) or to a specific response (higher levels of antibody to one or two types). To alleviate this drawback, we measured antibody to six individual polysaccharide types in a subset of sera. The data in Tables 1 and 2 showed that we did not observe a broadening of the antibody response with increasing age in the sense that more and more serotypes were recognized. This would be expected if the antibody response reflects the exposure to different pneumococcal serotypes. We previously observed just such an effect for antibodies to rotavirus (3, 6) and *E. coli* (2) in children. This lack of correlation of antibody response and exposure to *S. pneumoniae* was already observed in other studies. In a prospective study, no difference in antibody specificity was found at 12 and 24 months of age between carriers and noncarriers of specific pneumococcal serotypes (13). An analysis of acute- and convalescent-phase sera from children with otitis media due to *S. pneumoniae* revealed a poor antibody response in the first year of life: only about 10% of the infants showed a serum antibody response to the infecting pneumococcal serotype. This rate increased gradually to 35 and 47% in the second and third years of life, respectively (26).

IgM antibodies are generally the first to appear in an immune response and are later supplanted by IgG antibodies. Interestingly, IgM antibodies to pneumococcal polysaccharides were found with a prevalence of >50% in all age groups older than 2 years. Therefore, the presence of these antibodies cannot serve as a criterion for primary infections; they might instead indicate repeated infections with different serotypes of *S. pneumoniae*. Remember that we had previously observed a high prevalence of IgM antibody to rotavirus and lipopolysaccharide of enterotoxigenic *E. coli* in Ecuadorian children older than 2 years (3, 4).

In summary, a seroprevalence study like ours is unlikely to reveal true exposure to different *S. pneumoniae* serotypes. It reflects more the developmental maturation of the serum antibody response to pneumococcal polysaccharides and might thus be helpful for planning vaccination strategies. Notably, we observed a strong exposure-related antibody response to a different bacterial polysaccharide antigen, namely, the lipopolysaccharide of *E. coli*, in the same Ecuadorian children at 6 to 12 months of age (4). It would be interesting to analyze whether the different chemical structure or organ exposure (intestinal versus respiratory tract), or an adjuvant effect present in lipopolysaccharides but not in pneumococcal polysaccharides, makes lipopolysaccharides more immunogenic than pneumococcal polysaccharides for young children.

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