Standard case management of pneumonia in hospitalized children in Uruguay, 1997 to 1998

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Objective. To report the results of the use of antimicrobial guidelines for the management of children with community-acquired bacterial pneumonia.

Methods. Admittance and discharge criteria and algorithms for diagnosis and treatment were established. The decision to treat with antibiotics was based on radiologic findings in pneumonia with pulmonary consolidation and left to the attending physician’s criteria in the remaining cases. The use of antibiotics was limited to penicillin and derivatives (ampicillin, amoxicillin) and macrolides.

Results. Of the 1163 children treated as bacterial pneumonia, hospitalized in public and private health facilities in Montevideo from September, 1997, through September, 1998, standard case management was applied in 1082 (93%). Age distribution was: <1 month, 1%; between 1 and 11 months, 29%; between 1 and 5 years, 50%; >5 years, 20%. Chest radiography showed evidence of pulmonary consolidation in 843 children (73%). Bacteria were detected in blood culture and/or pleural fluid of 57 children (5%). In 51 the identified microorganism was Streptococcus pneumoniae, susceptible to penicillin in 30, intermediate in 6 and resistant in 5 (maximum MIC, 4 μg/ml); in 10 cases etiologic diagnosis was made by antigen detection. Empyema was present in 62 children (5.3%); 38 (3.27%) required treatment in an intensive care unit; and 5 (0.4%) died.

Conclusions. Compliance with standard case management was highly satisfactory. Outcome of children treated with penicillin and derivatives was good, including children with empyema and pneumatocele and two patients with penicillin-resistant S. pneumoniae. At the present time S. pneumoniae resistant to penicillin is not an important problem in children with pneumonia in Uruguay. Surveillance of identified microorganisms and their antimicrobial susceptibility must continue.

INTRODUCTION

Bacterial and viral pneumonia in children is an important cause of morbidity and mortality throughout the world, especially in developing countries. Uruguay is a small country of 3 million people, one-half of whom live in the capital city, Montevideo. Twenty-two percent are children 14 years old. In 1997 infant mortality was 16.3/1000 live births. Acute respiratory infections are the second cause of death in children 1 month to 1 year old and the third in children 1 to 5 years old. Pneumonia is also the most common cause of hospitalization in pediatric facilities during winter.

In 1994 Haemophilus influenzae type b conjugate vaccine was included in the free obligatory vaccination program with Calmette-Guerin bacillus, diphtheria-tetanus toxoids-pertussis, polio and measles-mumps-rubella vaccines. The WHO/Pan American Health Organization (PAHO) program for the control of acute respiratory infections is being applied.

Bacterial pneumonia poses the problem of choosing the proper antibiotic because of the possibility of drug resistance. Six percent of invasive strains of Haemophilus influenzae type b were beta-lactamase-producing in 1993 but are no longer a problem in our country in well-immunized children. In Uruguay Staphylococcus aureus is rarely isolated in children with pneumonia. As in the rest of the world there are increasing reports of invasive strains of Streptococcus pneumoniae with reduced susceptibility to penicillin and cephalosporins. The number of strains with some degree of penicillin resistance rose from 3% in...
1992 to 29% in 1994 and to 40% in 1996. In parallel with the rise in penicillin insensitivity, in 1996 ~20% of isolates also showed reduced susceptibility to third generation cephalosporins.

The clinical presentation of patients with pneumococcal pneumonia is not related to penicillin susceptibility. Furthermore several investigators have shown that beta-lactams are effective for treatment of children with pneumococcal pneumonia caused by strains with reduced susceptibility to penicillin. The use of antibiotics could be partly responsible for further enhancing the percentage of resistant strains, increasing the cost of treatment without benefit.

Although implications of resistant strains of *S. pneumoniae* in the treatment of pneumonia have not been fully elucidated, reports of an increasing number of these strains in our country led to irrational and inappropriate use of antimicrobials in this disease. Community-acquired pneumonia in children was treated in both public and in private institutions with a wide variety of antibiotics (ampicillin, aminoglycosides, second and third generation cephalosporins, macrolides, vancomycin). Alternating antibiotics without rational justification in the same patient during the course of the disease was also frequent. This irrational use of antibiotics could be partly responsible for further enhancing the percentage of resistant strains, increasing the cost of treatment without benefit.

Confronted with this problem the Sociedad Uruguaya de Pediatría (SUP) organized a multidisciplinary group to develop guidelines for the workup and treatment of community-acquired pneumonia. It was expected that the application of these guidelines would help to control the increasing trend of resistant strains of *S. pneumoniae* and at the same time would help to control the costs of health care of these patients.

We report the results of the application of these guidelines in the treatment of community-acquired bacterial pneumonia in hospitalized children during the first year of the project.

**METHODS**

The guidelines for standard case management of hospitalized children with community-acquired pneumonia were developed by a group of pediatricians, microbiologists, pulmonologists and infectologists from various public (Pereira Rossell Children’s Hospital, Military Hospital, Police Hospital) and private (Asociación Española 1ª de Socorros Mutuos and Centro de Asistencia del Sindicato Médico del Uruguay) institutions and from the Pulmonology and Infectology committees of the SUP.

Guidelines were based on available national data related to bacterial isolates from hospitalized children with community-acquired pneumonia, antimicrobial susceptibility and clinical presentation and outcome.

Criteria for diagnosis, hospital admittance and discharge were defined. Diagnostic criteria for pneumonia in children <5 years of age were those recommended by WHO/PAHO. Classic clinical and radiologic findings were used for older children. Hospital admittance criteria were: subcostal retractions; pleural effusion; hypoxemia (saturation <95%); conditions affecting respiratory function; access to ambulatory health care not easily available.

On admittance chest radiography and complete blood counts were performed, and a single blood sample was drawn for culture. When pleural effusion required thoracocentesis, Gram stain, detection of bacterial antigens and culture of pleural fluid were performed, as well as evaluation for protein, total differential cell counts, lactic acid dehydrogenase and glucose. Cultures, identification of isolated agents and susceptibility to specific antimicrobial drugs were performed by standard microbiology techniques. The MIC was determined by E-test. For penicillin *S. pneumoniae* strains with a MIC of ≤0.06 μg/ml were considered susceptible, strains with a MIC of 0.12 to 1 μg/ml were considered intermediate and those with a MIC ≥2 μg/ml were considered resistant. For cefotaxime or ceftriaxone strains with a MIC of ≤0.5 μg/ml were considered susceptible, those with a MIC of 1 μg/ml were considered intermediate and those with a MIC of ≥2 μg/ml were considered resistant.

Radiologic findings were interpreted by the attending pediatrician. Children with pulmonary consolidation or pleural effusion on chest radiography were treated as bacterial pneumonia with penicillin and derivatives (amoxicillin, ampicillin). If *Chlamydia* spp. or *Mycoplasma pneumoniae* were suspected, children were treated with macrolides (erythromycin, clarithromycin, azithromycin). Infants <1 month of age received ampicillin plus cefotaxime (Fig. 1).

Management of pleural effusion followed recommendations previously agreed on with the Department of Pediatric Surgery. After thoracocentesis surgical drainage was performed when at least two of the following were present: pH <7; glucose <0.4 g/dl; lactate dehydrogenase >1000 units; polymorphonuclear leukocytes; bacteria in Gram-stain smear.

Poor outcome of pneumonia was defined as two or more of the following: ill appearance; impairment of respiratory function (diagnosed by oxygen saturation or blood gases); increasing tachypnea or retractions; persistence of fever (axillary temperature >38.5°C) beyond the third day of treatment; and appearance of signs of pleural effusion.

Criteria for discharge were apyrexia for 48 h, improvement of general appearance and improvement or disappearance of tachypnea and retractions.

Patients were followed prospectively. Data were entered onto a form specifically designed for this study and included the following information: age; gender; history of immunodeficiency; date of admittance; radio-
logic findings; microbiologic findings; compliance with standard treatment; complications; need for intensive care; duration of hospitalization; death.

$P$ values were calculated by chi square or by Fisher's two tailed exact test. Calculations were performed with the Epi-Info statistical program (Version 6.0; CDC, Atlanta, GA).

RESULTS

In September, 1997, standard case management began to be applied in the city of Montevideo in children admitted to public (Pereira Rossell Children's Hospital, Military Hospital, Police Hospital) and private (Centro de Asistencia del Sindicato Médico del Uruguay and Asociación Española 1ª de Socorros Mutuos) health care centers.

From September, 1997, to September, 1998, 1163 children hospitalized in these health care centers were treated as bacterial pneumonia. Proposed standard case management was applied in 1082 children (93%). Cases were more frequent during winter and spring months.

Eighty percent of the children were $<5$ years old ($<1$ month = 14; 1 to 11 months = 338; 1 to 5 years = 577); 650 (56%) were males. One child had IgA immunodeficiency; none had a history of HIV infection.

Chest radiography showed evidence of pulmonary consolidation in 843 children (73%). Pulmonary consolidation was significantly more frequent in children $>1$ year of age. Identification of bacterial agents was independent of age (Table 1).

Bacteria were identified in blood culture and/or pleural fluid of 57 children (5%); $S$. pneumoniae in 51 (by culture in 41 and only by antigen detection in 10); $H$. influenzae type b in 3 children who were not vaccinated; $Staphylococcus aureus$ in a child with varicella pneumonia; $Pseudomonas aeruginosa$ in a child who probably had cystic fibrosis; $Streptococcus pyogenes$ in one child.

Of the $S$. pneumoniae isolates, 30 strains were susceptible, 6 were intermediate and 5 were resistant to penicillin. The highest MIC was 4 $\mu$g/ml and was found.

### TABLE 1. Pulmonary consolidation, pleural effusion and identification of bacteria by age group

<table>
<thead>
<tr>
<th>Status</th>
<th>Age $&lt;1$ yr ($n = 352$)</th>
<th>Age $&gt;1$ yr ($n = 811$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary consolidation</td>
<td>184 52</td>
<td>659 81</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>12 3.4</td>
<td>192 12</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Identification of bacteria in blood and/or pleural fluid</td>
<td>13 3.7</td>
<td>44 5.4</td>
<td>NS</td>
</tr>
</tbody>
</table>
in one strain. Table 2 compares *S. pneumoniae* susceptibility to penicillin and to third generation cephalosporins. The highest MIC for third generation cephalosporins was 2 μg/ml.

In the course of their illness 114 (10%) had pleural effusion, 62 of which were empyema; 41 (3.5%) also developed pneumatocele and 38 (3.27%) required treatment in the intensive care unit. Pleural effusion was significantly more frequent in children >1 year of age (Table 1).

Five children (0.4%) died. *S. pneumoniae* was identified in two of them. One of these patients, who was 8 months old, was admitted with pulmonary consolidation, empyema and pneumatocele. He developed hemolytic-uremic syndrome during the first 24 h, required mechanical ventilatory assistance and peritoneal dialysis and died after 31 days in hospital. He received ceftriaxone plus vancomycin; *S. pneumoniae* was susceptible to penicillin. The other patient, 2 years old, became ill 6 days before admittance and was evaluated by a physician on five occasions during that time. On admittance he had severe pneumonia with respiratory failure and empyema; he required mechanical ventilatory assistance, developed a pneumothorax and died during the first 24 h. He received vancomycin; *S. pneumoniae* was resistant to penicillin (MIC = 4 μg/ml). In the other three children without bacteria detected, one was 3 months old. Respiratory syncytial virus antigens were identified in a nasopharynx aspirate on admittance and death occurred after 32 days of hospitalization in the general ward. Another patient, who was 2 years old, presented with pneumonia and empyema, drained without incident. On the third day the patient showed progressive neurologic disorders indicative of diffuse brain injury, leading to deep coma and death. Autopsy revealed resolution of pneumonia and lesions compatible with purulent disseminated acute encephalomyelitis. The last patient, who was 10 months old, had respiratory symptoms in the 15 days before admittance. Chest radiography showed air trapping and diffuse interstitial infiltrates. After receiving oral amoxicillin for 9 days together with steroids and bronchodilators, symptoms became more severe. A new chest radiograph was interpreted as “pneumonitis.”

**TABLE 2. Streptococcus pneumoniae susceptibility to penicillin and to third generation cephalosporins (n = 51)**

<table>
<thead>
<tr>
<th>Third Generation Cephalosporin Susceptibility</th>
<th>Penicillin Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible ≤0.06 μg/ml (n = 30)</td>
<td>23 1 2</td>
</tr>
<tr>
<td>Intermediate 0.12–1.0 μg/ml (n = 6)</td>
<td>0 4 0</td>
</tr>
<tr>
<td>Susceptible ≥2 μg/ml (n = 5)</td>
<td>0 0 2</td>
</tr>
<tr>
<td>No data (n = 10)</td>
<td>7 1 1</td>
</tr>
</tbody>
</table>

The choice of the antibiotic for the treatment of suspected bacterial pneumonia is generally decided before the etiologic agent is known. This decision is based on the knowledge of local bacterial agents causing the disease and their antimicrobial susceptibility.
In countries like Uruguay where *H. influenzae* type b conjugate vaccine is included in the vaccination program, *S. pneumoniae* is the most frequent cause of bacterial pneumonia. Treatment of pneumococcal pneumonia is being evaluated. There is evidence that penicillin or derivatives are effective when MIC is >2 μg/ml. Some authors advise this antibiotic therapy. However, there are some published cases of therapeutic failure in pneumococcal pneumonia caused by strains with MIC >4 μg/ml.

There are no difference on clinical presentation of children with pneumonia caused by penicillin-susceptible and penicillin-resistant strains.

There are few recent publications on the outcome for children with suspected bacterial pneumonia without known etiology receiving empirical treatment with penicillin and derivatives. Most of the studies refer to children with confirmed pneumococcal pneumonia who received many antibiotic regimens. It is therefore difficult to compare the overall results of the application of this standard case management to other treatment strategies. Nevertheless mortality (0.4%), need for intensive care (3.2%) and complications (10%) were similar to those reported by other authors.

Acceptance of the proposed standard case management in the participating institutions, evidenced by application in 93% of the children treated as bacterial pneumonia, was highly satisfactory. The commitment of the people responsible for these services strongly contributed toward the compliance with the standard, which constituted invaluable support for young physicians making decisions regarding the treatment of their patients with pneumonia.

Bacterial etiology was confirmed in only 5% of the children. It is possible that many of these cases treated as bacterial pneumonia were of viral etiology; therefore their course was independent of the use of antibiotics. The high percentage of pneumonia with pulmonary consolidation (73%) and pleural effusion (10%) indicates otherwise. The possibility of viral etiology in this group of patients seems higher in children <1 year old, in whom pulmonary consolidation and pleural effusion were significantly lower. The implementation of rapid viral identification techniques would allow optimization of the application of this standard case management. Meanwhile, limiting the use of antibiotics to penicillin and macrolides during empiric treatment seems both appropriate and desirable.

Isolation of bacteria from blood cultures in children with pneumonia is a problem worldwide. In an effort to improve the recovery of microorganisms, increasing the number of samples drawn for blood culture before starting antibiotic treatment in children with severe disease and appropriate handling of the samples are necessary.

Bacterial agents identified in blood and pleural fluid were similar to those in previous findings on which these guidelines were based: *S. pneumoniae* was the main agent of community-acquired pneumonia; *Staphylococcus aureus* was an exceptional finding; and *H. influenzae* type b was found in only a few children who had not been vaccinated.

In this group of patients *S. pneumoniae* with reduced susceptibility to penicillin was less frequent than previously described in our country. Most of the strains of *S. pneumoniae* were susceptible to penicillin, and among resistant strains (12%) the highest MIC value obtained was 4 μg/ml. Some authors suggest that considering the concentration of third generation cephalosporins reached in serum and pleural fluid, pneumonia caused by *S. pneumoniae* with MICs of up to 4 μg/ml for third generation cephalosporins may be successfully treated with these antimicrobials. This seems suitable for our patients.

Initial empiric treatment with penicillin and derivatives in this group of children with community-acquired pneumonia was appropriate as judged by...
their overall outcome. In only one of the children who died, death could be attributable to pneumonia. This child was admitted after 6 days of illness with respiratory failure and empyema; resistant *S. pneumoniae* was isolated from blood and pleural fluid and he received vancomycin as the initial antibiotic treatment. Children with empyema and pneumatocele were successfully treated according to standard case management. In most of these children etiology remained unknown.

The microbiologic findings, together with the favorable response to treatment with penicillin and derivatives, confirm the rationale of the proposed standard case management and are a stimulus to continue its application. If the current epidemiologic situation is maintained, third generation cephalosporins will be the first alternative when penicillin-resistant *S. pneumoniae* is suspected and has not been identified. Third generation cephalosporins will also be used in the presence of *S. pneumoniae* with a penicillin MIC of $\geq 4$ μg/ml and a third generation cephalosporin MIC of $\leq 4$ μg/ml.

Children in whom bacterial agents were identified ($n = 51$) were more severely ill: they had higher frequency of pleural effusion, empyema and pneumatocele; they required intensive care more frequently and their mortality was higher. More severe pneumonia is associated with a greater frequency of bacteremia, which explains why the agent could be identified in these children. Identification of a bacterial agent was independent of the age of the patients. Mortality of these children with bacterial pneumonia is closer to figures of developed countries than to those of developing countries. Compliance with the proposed standard case management and results of its applications were highly satisfactory. The program will continue and will be periodically evaluated.

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Pneumococcal nasopharyngeal colonization in young South Indian infants

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Background. *Streptococcus pneumoniae* is the most frequent bacterial cause of morbidity and mortality in young children. Bacteria carried in the nasopharynx of healthy children reflect the prevalent strains circulating in the community.

Methods. We recruited 464 newborns from a rural area in South India with endemic vitamin A deficiency. Nasopharyngeal specimens were collected from each infant at ages 2, 4 and 6 months.

Results. Fifty-four percent of study infants were colonized by age 2 months, with 64.1 and 70.2% carriage prevalence at ages 4 and 6 months, respectively. The odds of carriage at 2 months were significantly increased in female infants, infants living in a household in which 20 or more cigarettes were smoked each day, infants whose mothers had less than 1 year of schooling and infants fed colostrum. At age 4 months infants having 2 or more siblings <5 years of age were at significantly increased risk of carriage. At age 6 months none of the potential risk factors examined achieved statistical significance, but maternal night blindness increased the risk of colonization 3-fold. The odds of carrying a PncCRM<sub>197</sub> vaccine serotype were increased among infants born to mothers who experienced night blindness during pregnancy. The most prevalent serogroups/types during the first 6 months of life were 6, 9, 10, 11, 14, 15, 19, 23 and 33, accounting for 76.7% of all serotyped isolates.

Conclusions. South Indian infants experience high rates of pneumococcal carriage during the first 6 months of life, which may partially explain their increased risk for pneumonia.

INTRODUCTION

In developing countries *Streptococcus pneumoniae* infection is the most frequent cause of bacterial pneumonia in young children and is a leading cause of death among infants. In addition to pneumonia pneumococci are important causes of otitis media, sepsis and meningitis. The nasopharynx is the primary reservoir for pneumococci in the body. Pneumococci carried in the nasopharynx of healthy children reflect the strains that circulate in the community and cause respiratory infections. Early and abundant colonization, coupled with immature immune systems, puts infants at risk for pneumococcal disease. Children acquiring a new strain develop disease ~15% of the time, and disease occurs most often within 1 month of acquiring a new serotype.

Two new pneumococcal conjugate vaccines that target infants are potentially useful in less developed countries: the 9-valent PncCRM<sub>197</sub> (Wyeth Lederle Pediatrics and Vaccines); and the 11-valent PncTD (Aventis Pasteur) pneumococcal conjugate vac-
The potential impact of these new vaccines will largely depend on the proportion of infections caused by serotypes included in the vaccine. Region-specific epidemiologic data are, therefore, crucial to the formulation of an effective pneumococcal conjugate vaccine because the prevalence of the serotypes associated with invasive disease varies temporally, geographically and by age. \cite{11,12}

For India, the country with the world’s largest population of children <5 years of age, there is little information on the distribution of serotypes and the risk factors for pneumococcal infections. \cite{10} Suspected cases of pneumonia are treated empirically; blood cultures are not routinely collected and have little value in areas where antibiotic use before seeking medical care is common.

To determine whether the conjugate vaccines are likely be effective in India, we prospectively studied the epidemiology of pneumococcal nasopharyngeal (NP) colonization and the distribution of pneumococcal NP serotypes in South Indian infants during their first 6 months of life. We describe the frequency of NP carriage of culture-positive pneumococci, risk factors for colonization and the distribution of pneumococcal serotypes at 2, 4 and 6 months of age. In addition we examine the risk factors for colonization with serotypes included in the PncCRM\textsubscript{197} pneumococcal conjugate vaccine. \cite{9,10}

**METHODS**

**Study population.** The Infant Pneumococcal Acquisition/Carriage in Tamilnadu (InPACT) study was nested within an ongoing, randomized, double blinded, placebo-controlled Vitamin A Supplementation in Newborns (VASIN) trial. The objectives of VASIN are to evaluate the effect of vitamin A given at birth on mortality, morbidity and growth in 9000 newborns through the first 6 months of life. The 3-year trial, which began on August 13, 1998, is being conducted in Natham and Karriyapatty, 2 rural blocks in the southern Indian state of Tamilnadu. These areas were selected because they have endemic vitamin A deficiency, show a high incidence of acute respiratory infection and share demographic similarities with other rural communities in South Asia.

**VASIN enrollment.** All pregnant women are eligible for enrollment in VASIN. Enrollment is strictly voluntary. Once data on household characteristics are collected, an enrollee is then randomized to have her newborn receive either two 24 000-IU vitamin A capsules or placebo within 48 h of birth. At delivery an infant’s vital status, sex and weight are noted before treatment. Morbidity assessments are conducted every 15 days during the follow-up period.

**InPACT study enrollment.** From October 22, 1998, through June 30, 1999, nasopharyngeal specimens were obtained from 464 infants. All infants 2 to 2.5 months old who were born into the VASIN trial and lived in Natham were eligible for enrollment in the InPACT study. Natham was selected because it had logistic advantages over Karriyapatty, including a larger and denser population.

**Ethical review.** The study protocols were approved by the Ethical Committee of the Aravind Center for Women, Children and Community Health, the Lions Aravind Institute for Community Ophthalmology and the Committee on Human Research of the Johns Hopkins University School of Hygiene and Public Health. An ethical committee constituted by the government of Tamilnadu also approved the protocols. Infants were included in each study on the basis of oral informed consent from parents or guardians. Verbal consent was appropriate in this community because the level of literacy is low.

**Data collection.** Nasopharyngeal specimen collection. Nasopharyngeal specimens were collected from each infant at three times, at enrollment (2 months of age) and then again at ages 4 and 6 months. Infants for whom swabs had not been collected between 2 days before and 14 days after each infant’s 2-, 4- or 6-month birthdays were classified as “missed.” Specimens were obtained following a set protocol. A small, flexible rayon-tipped swab (DIFCO CultureSwab Transport System with Amie’s medium) was inserted into one of the nares to the level of the posterior nasopharynx. The swab was left in place for 5 s or rotated 180 degrees before removal. Swabs were then inserted into Amie’s transport media and transported to the microbiology laboratory at the Aravind Eye Hospital within 10 h of collection.

**Laboratory procedures.** At the laboratory swabs were inoculated onto blood agar (Becton Dickinson) plates containing 5% sheep blood and 2.5 mg/l gentamicin (Nathan Pirumal, Bombay, India). Plates were incubated at 37°C in 5% CO\textsubscript{2} for 18 to 24 h. Colonies exhibiting classic pneumococcal morphology were confirmed by optochin (Taxo) inhibition or bile solubility testing. Pneumococcal reference strains (American Type Culture Collection 5603) were used for quality control. Isolates were serogrouped/typed using PNEUMOTEST kits (Staten Seruminstitut, Copenhagen, Denmark). The antisera included in the kits reacts with serotypes 1, 2, 3, 4, 5, 8, 14 and 20 and with serogroups 6, 7, 9, 11, 12, 15, 17, 18, 19, 22, 23 and 33. We also identified the specimens that contained at least one of the serogroups/types in the nine-valent PncCRM\textsubscript{197} vaccine. \cite{10} This vaccine includes serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23 F.

**Data management and statistical analysis.** Bivariate associations between each potential risk factor and colonization at 2, 4 and 6 months of age were...
determined by two tailed chi square tests or Fisher's exact tests, where appropriate (Stata 6.0; Stata Corp., College Station, TX). Logistic regression models were constructed to identify risk factors for colonization at each age: 2, 4, and 6 months. The potential risk factors that were statistically significant at the 0.10 level in bivariate analysis for at least one of the time points were entered into each of the three models. Similar models were constructed for colonization with any PncCRM197 serogroups/types. Because the VASIN data showed that vitamin A supplementation was associated with a modest reduction in carriage, regression models include receipt of vitamin A as a covariate. Associations between potential risk factors and colonization in the multivariate models were considered to be statistically significant at \( P \leq 0.05 \). It is likely that the observed colonization prevalence in each age group underestimated the true colonization incidence because of the dynamics of pneumococcal colonization, given that the samples were collected at 2-month intervals. Therefore we used odds ratios rather than relative risk estimates to characterize the association between potential risk factors and colonization.

RESULTS

Enrollment and follow-up. We identified 539 infants thought to eligible for the InPACT trial. At the time of the InPACT enrollment visit at age 2 to 2.5 months, 11 (2.0%) had died and 63 (11.7%) had migrated. The parents of one child refused enrollment. For the 464 infants enrolled at age 2 months, follow-up rates at ages 4 and 6 months were 87.5% (406 of 464) and 77.3% (359 of 464), respectively (Fig. 1).

Demographic characteristics. Most of the families were Hindu and were members of marginalized communities (Table 1). More than one-half of the study infants were male, and almost one-third of the infants weighed \( \leq 2500 \) g at birth. Female infanticide is an issue in rural South India and may partially explain the gender disparity (43.3% female infants vs. 56.7%...
male infants) among our study infants. All study infants were breast-fed and >80% received colostrum. Approximately 10% of the mothers reported experiencing night blindness during pregnancy, a classic symptom of vitamin A deficiency. Nearly one-half of the infants came from homes where one or more cigarettes were smoked per day and wood was the primary cooking fuel in >94% of the households. The majority of infants (86.1%) had one or no sibling. The families of the infants were of low socioeconomic status. Fewer than one-half of the families owned a means of transportation, and nearly 50% of the mothers had no formal education.

**Prevalence of pneumococcal nasopharyngeal colonization.** By age 6 months *S. pneumoniae* were isolated at least once from the nasopharynx of 400 of the 464 children, a colonization rate of 86.2%. Children acquired pneumococci at an early age; 53.9% were colonized by age 2 months. Carriage prevalence among specimens Cultured at ages 4 and 6 months was 64.1 and 70.2%, respectively.

**Longitudinal colonization of the nasopharynx.** The pattern of longitudinal colonization of the 464 study infants is summarized in Table 2. Approximately 28% of the infants had 1 or more missing specimens. Among those with 3 specimens the most common single pattern of colonization (26.6%) was to acquire pneumococci at age 2 months and to stay colonized through the sixth month of life. Only 10.4% of the infants who were free of pneumococci at ages 2 and 4 months became colonized at age 6 months, and 9.0% of the infants were never colonized. Among those infants who were culture-negative at age 2 months, the probability of colonization at age 4 months was 58.3%. Children who were negative at ages 2 and 4 months had a 53.8% chance of being colonized at age 6 months and among those negative at age 4 months the risk of being colonized at age 6 months was 64.2%.

**Risk factors for nasopharyngeal carriage of pneumococci.** By bivariate analysis nine factors were associated with colonization at 2, 4 or 6 months of age (Table 3). Low birth weight, runny nose at specimen visit and number of health visits before specimen visit were not associated with colonization.

**Age 2 months.** In the multivariate analysis female infants, infants born to uneducated mothers and infants exposed to 20 or more cigarettes a day were at increased risk of a colonization. Feeding of colostrum was also a risk factor.

**Age 4 months.** Having more than one sibling younger than 5 years significantly increased the odds of colonization among infants [adjusted odds ratio, 2.39 (1.21, 4.72); *P* = 0.01]. No other variable included in the regression model was statistically associated (*P* = 0.05) with colonization at 4 months of age.

**Age 6 months.** By multivariate analysis none of the risk factors studied was associated with colonization at age 6 months. The odds of colonization among infants whose mothers had experienced night blindness during pregnancy were 3 times higher than infants born to mothers who did not experience night blindness, although the confidence interval was wide [adjusted odds ratio, 3.04 (0.61, 15.13); *P* = 0.18] and encompassed one.

**Distribution of serogroups/types.** In total 681 pneumococcal isolates were serogrouped/typed: 182 (26.7%) at 2 months; 253 (37.2%) from infants at 4 months; and 246 (36.1%) from infants at 6 months. The most prevalent serogroups/types among 2-month-old infants were groups/types 6 (11.0%), 9 (5.5%), 10 (6.0%), 14 (8.8%), 15 (7.7%), 19 (6.0%) and 23 (13.7%). The remaining serotypes were each responsible for <5% of the isolates. Among the 4-month-old infants, group/type 23 was isolated most frequently (18.9%).
Other prevalent serotypes were 6 (9.1%), 11 (5.1%), 14 (6.7%), 15 (8.3%) and 19 (11.1%). Similarly among infants age 6 months, the most prevalent serotypes were 6 (9.8), 9 (5.7%), 14 (5.3%), 15 (7.7%), 19 (12.6%), 23 (17.5%) and 33 (7.7%); these constitute 66.3% of serotypes carried by infants at age 6 months. The five most prevalent serogroups/types over the entire 6-month period were 6 (9.8), 14 (6.8%), 15 (7.9%), 19 (10.3%) and 23 (16.9%); in total these 5 serogroups/types accounted for 54.7% of the 681 isolates. There were 115 (17.1%) isolates for which the serogroup/type could not be determined.

Duration of colonization by serogroup/type. The duration of carriage was <2 months in the majority of serogroup/type-specific colonizations. Carriage lasting 2 months or more was observed in 8.1, 16.6, 2.2, 8.4, 17.0 and 16.6% of colonizations with serogroups/types 6, 9, 14, 15 and 23, respectively. The proportion of colonizations in which the same serogroup/type was detected in all three sampling periods was low. They represented 3.3, 4.2, 1.7 and 3.1% of colonizations with serogroups/types 9, 15, 19 and 23, respectively. None of the infants carried serotypes/groups 6 or 14 for three age intervals.

Risk factors for PncCRM197 serotype colonization. Four factors, birth weight, blindness, runny nose and season, were bivariately associated ($P \leq 0.10$) with colonization by any PncCRM$_{197}$ serogroups/types at 2, 4 or 6 months of age or cumulatively. Of these factors only maternal night blindness was associated with a 2.97 odds ratio of PncCRM$_{197}$ serogroup/type colonization in the multivariate analysis, although this did not reach statistical significance.

Distribution of PncCRM$_{197}$ serotypes. The 9 serogroups/types included in the PncCRM$_{197}$ vaccine accounted for 50.4% of the 681 isolates collected. The proportion of PncCRM$_{197}$ vaccine serogroups/types isolated at ages 2, 4 and 6 months was 51.7, 47.8 and 50.0%, respectively.

DISCUSSION

Young South Indian infants experience a very high prevalence of pneumococcal nasopharyngeal carriage. Evidence from other studies suggests that the duration of carriage with a pneumococcal strain ranges from 5 days to 17 months, and the same strains can be acquired and lost more than once.3, 14 Because we collected specimens at 2-month intervals, it is likely that the observed colonization prevalences underestimate the true colonization incidence. The observed carriage rates are comparable with those reported from other regions in the developing world.12 All infants in a 1986 Papua New Guinea study were colonized by age 3 months.14 Similarly in sub-Saharan Africa, up to 80% of children at 2 months of age were colonized.15, 16 In contrast 12% of children in Sweden were carriers by age 3.17 Children in the United States, on average, acquire their first strain at

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### TABLE 3. AOR and 95% confidence interval of potential risk factors for pneumococcal carriage in InPACT study infants ($n = 464$)*

<table>
<thead>
<tr>
<th>Potential Risk Factor</th>
<th>Age 2 mo</th>
<th>Age 4 mo</th>
<th>Age 6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>%</td>
<td>AOR</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>262</td>
<td>48.5</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>200</td>
<td>61.0</td>
<td>1.73 (1.17, 2.56)†</td>
</tr>
<tr>
<td>Infant fed colostrum?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>84</td>
<td>39.3</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>378</td>
<td>60.7</td>
<td>2.14 (1.30, 3.56)</td>
</tr>
<tr>
<td>History of night blindness during pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>421</td>
<td>53.4</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>41</td>
<td>58.5</td>
<td>1.09 (0.54, 2.17)</td>
</tr>
<tr>
<td>Total no. of cigarettes smoked/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>410</td>
<td>52.0</td>
<td>1</td>
</tr>
<tr>
<td>20 or more</td>
<td>52</td>
<td>69.2</td>
<td>1.91 (1.0, 3.68)</td>
</tr>
<tr>
<td>Fuel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood</td>
<td>437</td>
<td>36.0</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>25</td>
<td>54.9</td>
<td>0.66 (0.27, 1.59)</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry/cool</td>
<td>212</td>
<td>49.1</td>
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<tr>
<td>Humid/rainy</td>
<td>250</td>
<td>50.9</td>
<td>1.38 (0.94, 2.04)</td>
</tr>
<tr>
<td>Mother's years of education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or more</td>
<td>254</td>
<td>47.2</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>208</td>
<td>62.0</td>
<td>1.72 (1.15, 2.57)</td>
</tr>
<tr>
<td>Conveyance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicycle</td>
<td>185</td>
<td>47.0</td>
<td>1</td>
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<tr>
<td>None</td>
<td>277</td>
<td>52.8</td>
<td>1.48 (0.99, 2.20)</td>
</tr>
<tr>
<td>Siblings &lt;5 yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or none</td>
<td>397</td>
<td>53.2</td>
<td>1</td>
</tr>
<tr>
<td>2 or more</td>
<td>65</td>
<td>58.5</td>
<td>1.06 (0.61, 1.85)</td>
</tr>
</tbody>
</table>

* Adjusted for the effect of vitamin A.
† Numbers in parentheses, 95% confidence interval.
age 6 months. In Israel, a transitional society, ~26% of infants are colonized by age 2 months. The varying rates suggest that infant acquisition and carriage of pneumococci are largely influenced by socioeconomic factors. This hypothesis is corroborated by studies in the United States, Israel and Australia that found higher rates of colonization and disease among marginalized minority populations than in the general population.

Infant’s gender (being female), having two or more siblings under age 5, lack of maternal education and exposure to 20 or more cigarettes per day were associated with early colonization. However, these factors had little effect on whether a child was colonized with PncCRM197 vaccine serotypes. Although a few risk factors have the potential for modification, real differences in socioeconomic status are small in this population when viewed on a macroscopic level. Therefore it is unlikely that risk factor modification alone will have a significant impact on risk of pneumococcal disease.

Infants whose mother’s experienced night blindness during pregnancy were more likely to be colonized with pneumococci. We are unaware of other studies that have looked at this association. There is some evidence to suggest that vitamin A deficiency can increase bacterial adherence to the respiratory tract, which in turn increases the risk of bacterial colonization, and infants of mothers who were night-blind during pregnancy have been shown to be more likely to die in the first 6 months of life.

In addition we found unexpectedly that colostrum-fed infants were more likely to be colonized at 2 months than those from whom colostrum was withheld. It is possible that the association between the colostrum and colonization is confounded by unidentified factors and warrants further investigation.

The 10 serotypes/serogroups that were most prevalent throughout the first 6 months of life comprised 73.3% of all isolates. The prevalence of these 10 serotypes was similar across age groups, although the rank order was different. In most instances the duration of carriage with a specific serotype/group was <2 months, an indication that the majority of colonizations represent strains that are new or strains that have been reacquired rather than long term carriage of the same strain. Carriage of short duration is correlated with incidence of pneumococcal infection.

The 9-valent PncCRM197 conjugate vaccine protects against 50% of all the serogroups/types identified among the South Indian infants. The 11-valent PncTD vaccine, which contains the same 9 antigens with the addition of serogroups/types 3 and 7F, protects against an additional 3% of the identified serogroups/types. If there is cross-protection within serogroups, each of these vaccines would reduce colonization by ~50%. The level of protection conferred by these vaccines may be higher. It is possible that our 2-month sampling intervals missed capturing those serotypes that colonize for a shorter duration. In addition individuals can be colonized with up to four serotypes at any given period; we serotyped only the predominant strains colonizing the nasopharynx.

Data from 6 Indian medical referral centers identified invasive isolates collected from children <5 years of age hospitalized for severe pneumococcal disease as 1,4, 5, 6, 7, 12, 14, 16, 18, 19 and 45. If these 11 serogroups/types, which accounted for 32% of the isolates in our population, are responsible for the majority of disease in our region, then the vaccines would protect against ~25% of the strains seen in our population.

Because pneumococcal colonization of the nasopharynx among South Indian children occurs in early infancy and the risk factors identified for pneumococcal colonization are not easily modified, vaccination might prevent infant morbidity and mortality from pneumococcal disease.

ACKNOWLEDGMENTS

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gastroenteritis in Finnish infants

XIAO-LI PANG, MD PHD, SHANG-QIN ZENG, MD, SHINJIRO HONMA, MD, SHUJI NAKATA, MD, PHD AND TIMO VESIKARI, MD, PHD

Effect of rotavirus vaccine on Sapporo virus gastroenteritis in Finnish infants

Background. Sapporo-like viruses (SLVs) occur worldwide, but there is limited information about the SLV-associated gastroenteritis outside Japan.

Methods. Stool specimens from 1432 episodes of gastroenteritis that occurred in children between 2 months and 2 years of age during a rotavirus vaccine trial (776 episodes in placebo-vaccinated and 656 in rotavirus-vaccinated infants) were examined for SLVs using a reverse transcription-PCR assay. The reverse transcription-PCR took advantage of new primers specific for Sapporo virus genetic clusters I, II and III; SV/SV82 (SV/Sapporo virus 82); SV/Lond92 (SV/London 92); and SV/PV (Parkville virus).

Results. SLVs were detected in association with 132 (9.2%) of all episodes; in 80 (5.6%) episodes SLV was the only gastroenteritis virus detected. The epidemic season of SLVs peaked from March to May concurrently with rotaviruses and astroviruses and overlapping with...
Norwalk-like viruses. Clinically SLV gastroenteritis was characterized by a mild diarrheal disease, being sharply different from the Norwalk-like virus-associated “winter vomiting disease.” Rotavirus vaccination did not have any effect on the number of SLV episodes, but the intensity and duration of SLV-associated diarrhea were reduced in rotavirus-vaccinated children compared with placebo-vaccinated children ($P = 0.0008$).

**Conclusions.** SLVs are common causative agents of acute gastroenteritis in young Finnish children. SLV disease is characterized by diarrhea, which is usually mild but can be severe. By an unknown mechanism rotavirus vaccine seems to reduce the severity of SLV-associated diarrhea.

**INTRODUCTION**

Sapporo virus (SV) was originally identified by electron microscopy from diarrheal stools of infants in an infant home in Sapporo, Japan, and in several subsequent outbreaks of gastroenteritis in the same infant home, as detected more recently with newly developed reverse transcription (RT)-PCR methods (S Honma, S Nakata, Y Sakai, K Numata-Kinoshita, M Tatsumi, S Chiba. Sensitive detection and differentiation of Sapporo virus, a member of the family Caliciviridae, by standard and booster nested PCR, submitted for publication). Outside Japan Sapporo-like viruses (SLVs) have been detected in 2.9% of diarrheal stools of children attending day care in the US and in a small number of children hospitalized with gastroenteritis in South Africa. Serologic studies have suggested that infection with SLVs is common worldwide; thus there is a gap between identified and assumed cases of SLV infection.

We recently showed that Norwalk-like viruses (NLVs), which, like SLVs, also belong to Caliciviridae but are now regarded as a separate genus, are much more common (~20% in acute gastroenteritis in young children) than had been previously supposed; this finding helped to close a “pediatric gap” for NLVs. The keys to this observation were (1) examination of cases occurring in the community rather than those requiring hospital admission and (2) use of broadly reactive and sensitive consensus primers for the detection of NLVs. In an analogous manner, we have now extended the study to SLVs, with the use of primers for detection and differentiation of SV genetic clusters I, II and III; for SV/SV82 (SV/Sapporo virus 82); SV/Lond92 (SV/London 92); and SV/PV, respectively (S Honma, S Nakata, Y Sakai, K Numata-Kinoshita, M Tatsumi, S Chiba. Sensitive detection and differentiation of Sapporo virus, a member of the family Caliciviridae, by standard and booster nested PCR, submitted for publication).

In this article we report on the incidence and characterize clinical features of SLV-associated gastroenteritis in young Finnish children and show that an oral rotavirus vaccine may have an ameliorating effect on diarrhea caused by SLVs.

**MATERIALS AND METHODS**

**Patients.** The conduct of the rotavirus vaccine trial and collection of stool specimens from episodes of gastroenteritis have been described previously. Briefly this was a randomized double blind placebo-controlled efficacy trial of rhesus-human reassortant rotavirus tetravalent (RRV-TV) oral vaccine. Enrollment began in September, 1993, and ended in September, 1994, and the follow-up for gastroenteritis was continued until June, 1995. On enrollment the children were 2 to 3 months of age, and at the end of the study they were between 12 and 24 months of age. Those enrolled early were in follow-up for two winter epidemic seasons of gastroenteritis, and those enrolled late were in the follow-up for only one winter epidemic season. Of the 2398 children recruited, 1191 received the RRV-TV vaccine, and 1207 received placebo vaccine.

The follow-up for gastroenteritis episodes was a combination of active and passive surveillance. When there were signs of gastroenteritis (e.g. looser than normal stools or vomiting), the parents of the participating children were instructed to collect a stool specimen, to start a diary on symptoms and to contact the study nurse. In the event of gastroenteritis the study nurses gave instructions on home treatment, but as a rule the case management in more severe cases went through the normal channels of the health care system (health care center or private physician→outpatient clinic of hospital→admission to hospital). Gastroenteritis was defined as at least three looser than normal stools within a 24-h period or an episode of forceful vomiting with any loose stools. All episodes that met the definition were graded according to severity with a 1- to 20-point score.

The stool specimens were initially stored at home in a refrigerator and collected daily for storage at ~20°C until tested. First rotavirus antigen was tested using an enzyme immunoassay, and the rotaviruses in enzyme immunoassay-positive stools were genotyped with RT-PCR. During this testing some of the stool specimens were exhausted. To start with stool specimens were available from 1561 episodes. Of these specimens 811 were tested for enteric adenoviruses and astroviruses, and 1472 were tested for NLV genogroups I and II. After that 1432 specimens were available for studies on Sapporo-like viruses.

**Laboratory methods.** Single stranded RNA of NLV and SLV was extracted from 50 µl of the 10% stool
RT-PCR for SLVs was established on the basis of the method of Honma et al. Primers SLV-r-cl and Sapp36 were used in the first PCR. This was followed by a nested PCR with again primer SLV-r-cl, together with primers SV-s1, SLVs2 and SLV-s3 for detection of three genogroups [I, Sapporo virus 82 (SV82) genogroup; II, London 92 (Lond 92) genogroup; and III, PV genogroup] of SLV. First 5 \( \mu l \) of the RNA solution were added to a 25-\( \mu l \) reaction mixture containing 10 mM Tris-HCl, 50 mM KCl, 3.5 mM MgCl\(_2\), 0.3 \( \mu M \) Sapp36 primer, 40 units of RNA inhibitor and 100 units of Moloney murine leukemia virus reverse transcriptase. For the RT reaction the mixture was incubated for 1 h at 42°C, and 70 \( \mu l \) of PCR reaction mixture containing 10 mM Tris-HCl, 50 mM KCl, 3.5 mM MgCl\(_2\), 0.3 \( \mu M \) Sapp36 primer and 5 units of Taq polymerase were added. The reaction mixture was denatured at 94°C for 3 min and run for 35 cycles of 1 min at 94°C, 1 min and 20 s at 45°C, 1 min at 72°C and a final extension 15 min at 72°C. For the nested PCR, 5 \( \mu l \) of a 1/100 dilution of the first PCR product was transferred to a reaction mixture containing 0.3 \( \mu M \) concentrations of each SLV-r-cl and SLV-s-mix (SLV-s1, SLVs2 and SLV-s3) primers, 10 mM Tris-HCl, 50 mM KCl, 3.5 mM MgCl\(_2\) and 5 units of Taq polymerase. The cycling conditions were identical with those in the first PCR, except that 30 cycles were run and the annealing was at 60°C.

In the first PCR amplified products with 828 bp are produced. In the nested PCR amplified products with the expected different sizes (700 bp for the SV82 genogroup, 505 bp for the Lond 92 genogroup and 608 bp for the PV genogroup) are detected.

All PCR-positive products of SLV were confirmed by Southern hybridization. The hybridization assay for confirmation of SLV was the same as that described for NLV\(^8\) except for different probes.

RESULTS

Of the 1432 stool specimens examined 132 (9.2%) were positive for SLVs. SLVs were identified as the only viral pathogen in 80 (5.6%) cases; thus in the remaining 52 (3.0% total, or 39% of SLV-positive cases) one or more other gastroenteritis viruses were detected in the same stool specimen. The SLV-positive cases were equally divided between the RRV-TV vaccine and placebo recipient children, as were also the cases in which SLV was found as the only gastroenteritis virus (Table 1).

Of all detected SLVs 116 (88%) belonged to the SV/SV82 (SV/Sapporo virus 82) genetic cluster, and 13 (9.8%) belonged to the SV/Lond92 (SV/London 92) genetic cluster. In 3 (2.2%) cases both SV/SV82 (SV/Sapporo virus 82) and SV/Lond92 (SV/London 92) genetic cluster were found in the same specimen. The SV/PV genetic cluster was not detected among these cases.

There was a clear seasonality in the occurrence of SLV gastroenteritis, with a peak from March to May (Fig. 1). Coinfection with other gastroenteritis viruses peaked at the same time, as the season of rotaviruses and astroviruses coincided and that of NLVs overlapped with SLV season.\(^16\)

The clinical picture of SLV-associated gastroenteritis was typically a mild diarrheal disease with little vomiting. The median severity score on a 1- to 20-point scale was 6. However, there were also severe cases, as shown by the distribution of severity scores in pure SLV-associated gastroenteritis. Only two children (2%) with SLV-associated gastroenteritis were hospitalized.

Although the proportions of SLV-associated cases of gastroenteritis were similar in the rotavirus vaccine and placebo recipient children, there was a difference in the severity of diarrhea (but not vomiting): Those in the vaccine group had a shorter duration of diarrhea and a lower maximum frequency of diarrheal stools per 24 h (Table 2). On the 1- to 20-point scale those with a score of 11 or greater were regarded as severe.\(^10\) In the “pure” SLV-associated gastroenteritis there was one such case in the RRV-TV vaccine group vs. five in the placebo group (\(P = 0.153\), Fisher’s exact test, Fig 2).

DISCUSSION

This is the first study to illustrate the significance of SLV-associated gastroenteritis in young children in the community. To conclude SLVs appear to cause between 5 and 10% of gastroenteritis episodes in children <2 years of age. Although most cases are mild and therefore detectable only in a community-based study, some episodes are quite severe. Among the 162 episodes of gastroenteritis with a clinical score of 11 of 20 or greater (in the placebo group), SLVs accounted for 5 (3%).\(^16\)

In previous studies SLVs have only occasionally been detected outside Japan. The first finding was that of Matson et al.\(^5\) in an outbreak of gastroenteritis in a

<table>
<thead>
<tr>
<th>Viruses Detected</th>
<th>Vaccine</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLV only</td>
<td>36 (5.5)</td>
<td>44 (5.7)</td>
<td>80 (5.6)</td>
</tr>
<tr>
<td>SLV + rota</td>
<td>5 (0.8)</td>
<td>8 (1.0)</td>
<td>13 (0.9)</td>
</tr>
<tr>
<td>SLV + Ead</td>
<td>2 (0.3)</td>
<td>3 (0.4)</td>
<td>5 (0.3)</td>
</tr>
<tr>
<td>SLV + astro</td>
<td>3 (0.5)</td>
<td>8 (1.0)</td>
<td>11 (0.8)</td>
</tr>
<tr>
<td>SLV + NLV</td>
<td>9 (1.4)</td>
<td>4 (0.5)</td>
<td>13 (0.9)</td>
</tr>
<tr>
<td>SLV + rota + astro</td>
<td>3 (0.5)</td>
<td>0</td>
<td>3 (0.2)</td>
</tr>
<tr>
<td>SLV + rota + Ead</td>
<td>1 (0.1)</td>
<td>2 (0.3)</td>
<td>3 (0.2)</td>
</tr>
<tr>
<td>SLV + NLV + astro</td>
<td>1 (0.1)</td>
<td>1 (0.1)</td>
<td>2 (0.1)</td>
</tr>
<tr>
<td>SLV + rota + NLV</td>
<td>0</td>
<td>1 (0.1)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>SLV + rota + NLV + astro</td>
<td>0</td>
<td>1 (0.1)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Total positive for SLV</td>
<td>60 (9.1)</td>
<td>72 (9.3)</td>
<td>132 (9.2)</td>
</tr>
<tr>
<td>Negative for SLV</td>
<td>596 (90.9)</td>
<td>704 (90.7)</td>
<td>1300 (90.8)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percent.
  rota, rotavirus; Ead, enteric adenovirus; astro, astrovirus.
Houston day-care center. In Kenya 2.2% of gastroenteritis cases in children visiting outpatient clinics were found positive for Sapporo virus antigen in stools.4, 17 Classical human caliciviruses (SLVs) were found in a small number of adults (9 cases vs. 223 with Norwalk-like viruses) with gastroenteritis in Australia,18 and SLV genogroup III was found in an outbreak of gastroenteritis in Parkville, MD.19 In Japan SLVs have been found in up to 13% of children with gastroenteritis.4 In contrast serologic studies have shown that antibody prevalence to SLVs is between 70 and 100% in adult populations in different countries.4

The gap between the paucity of actually detected cases on one hand and the high seroprevalence on the other could be explained by use of insensitive diagnostic methods in previous studies or by common occurrence of subclinical SLV infections that pass unnoticed. Although subclinical cases have been found in previous studies,20 our findings clearly indicate that there are also a high number of mild symptomatic cases of SLV-associated gastroenteritis that are not brought to attention because they do not require medical intervention. Moreover the RT-PCR method applied in our study is more sensitive than previously used antigen detection tests for the diagnosis of SLV-associated gastroenteritis and therefore likely to reveal more cases.

We found that SLVs were present in 9.2% of all gastroenteritis episodes, but in only 5.6% of the episodes SLVs were detected as the sole pathogen. In the remaining cases other gastroenteritis viruses, i.e. rotaviruses, enteric adenoviruses, Norwalk-like viruses and astroviruses, were also detected by respective PCR methods.9, 12–14 This is not surprising because the epidemic seasons of all of these viruses, except enteric adenoviruses which occur year round, were in winter and spring.16 Moreover rotaviruses,22 and possibly other gastroenteritis viruses, can be excreted at low level and detected by RT-PCR for prolonged periods. Thus a virus from a previous infection can still be excreted at the time of a new infection by another gastroenteritis virus. Therefore it is not possible in each individual case to judge which of the simultaneously detected viruses is the actual causative agent of the episode in question. In any case the 5.6% of pure SLV-associated cases may be regarded as a minimum etiologic share in acute gastroenteritis attributable to SLVs.

The clinical picture in pure SLV gastroenteritis cases was one of diarrheal illness with little or no vomiting, in sharp contrast to the clinical picture of NLVs in the same child population.9, 16 The clinical characteristics of NLV disease in young children was more like “winter-vomiting disease,” with only a short course of diarrhea. Because of intensive vomiting the
overall clinical score in NLV disease (median, 8) was higher than in SLV disease (median, 6; \( P = 0.01816 \)).

The different clinical picture of SLV vs. NLV-associated gastroenteritis is compatible with the new classification of these viruses, which distinguishes SLVs (classical calicivirus morphology) as a separate genus within Caliciviridae, rather than as a genogroup of caliciviruses in humans. In this new classification NLVs (small round viruses) form another genus. SLVs and NLVs combined have an approximately 30% etiologic share of all cases of acute gastroenteritis in the community in Finnish children. This is about the same as the etiologic role of rotaviruses. Although rotaviruses as a rule cause more severe disease (median severity score, 11), the two genera of Caliciviridae form the second most important etiologic group in acute gastroenteritis in young children. SLVs appear to be of less clinical significance than NLVs. However, the full role of SLVs in gastroenteritis of children and adults requires much further study. Such investigations should be greatly facilitated with the application of the new RT-PCR detection methods.

The small but significant effect of RRV-TV rotavirus vaccine on pure SLV-associated diarrhea cannot be explained by specific immunologic protection induced by the vaccine, because coinfection with rotavirus was ruled out in the cases. However, the observation is analogous to our previous finding of adenovirus-associated diarrhea, which was also ameliorated by the RRV-TV vaccine. No effect of rotavirus vaccine was seen on NLV-associated cases, characterized by more vomiting than diarrhea. Therefore such a “nonspecific” effect of rotavirus vaccine seems to be limited to those cases of viral gastroenteritis in which diarrhea is the predominant clinical symptom.

REFERENCES

Non-type b Haemophilus influenzae disease: clinical and epidemiologic characteristics in the Haemophilus influenzae type b vaccine era

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Background. As a result of the decline in Haemophilus influenzae type b (Hib) disease caused by the widespread use of conjugate vaccines, non-type b H. influenzae will become a more important cause of H. influenzae (Hi) disease.

Characterization of the clinical and epidemiologic features of non-b Hi disease is needed in the Hib vaccine era.

Methods. A prospective active surveillance study of invasive Hi disease involving pediatricians in the United Kingdom and Republic of Ireland. For the first phase of the study (October 1, 1992, to October 31, 1995) pediatricians were asked to report any child who had invasive Hi disease and who had received Hib conjugate vaccine. For the second phase of the study (November 1, 1995. To December 31, 1998) pediatricians were asked to report any child with invasive Hi disease regardless of vaccination status.

Results. During the study period 102 cases of invasive non-type b Hi disease and 106 cases of invasive Hib disease were reported in children.
who had been fully vaccinated against Hib. Children with non-type b disease were younger (16 vs. 22 months of age, \( P = 0.08 \)), less likely to have meningitis and epiglottitis (\( P \leq 0.001 \)) and more likely to have pneumonia and bacteremia (\( P \leq 0.001 \)) than children with type b disease. For the last 2 years of the study invasive Hi disease occurring in a fully vaccinated child was more likely to be caused by a non-b strain than by a type b strain (58 vs. 38). In 1998 the incidence of non type-b Hi disease in all children \(<5\) years of age in the UK was 1.3/100 000 as compared with an incidence of Hib disease of 0.6/100 000. The majority (88%) of non-b strains isolated in children were nontypable strains.

Conclusions. Non-b Hi is a rare cause of disease in children, but in the Hib vaccine era it has become more common than type b as a cause of Hi disease in fully vaccinated children.

INTRODUCTION

_Haemophilus influenzae_ (Hi) was first identified as a pathogen in 1883 by Koch who described small Gram-negative rods in pus from patients with conjunctivitis.\(^1\) In 1931 Pittman\(^2\) identified nonencapsulated strains of Hi as well as six antigenically distinct encapsulated strains that could be identified serologically by characteristics of the polysaccharide capsule, now defined as serotypes a to f. She also recognized that one capsular type, serotype b, was responsible for almost all cases of Hi meningitis. Subsequently population-based studies have confirmed that _H. influenzae_ type b (Hib) is responsible for \(~95\%\) of all invasive Hi disease in unvaccinated children.\(^3\), \(^4\) The recent introduction of Hib conjugate vaccines has led to a substantial fall in the incidence of Hib disease in childhood.\(^5\)

With the decline in Hib disease, non-type b Hi have become a more important cause of Hi disease. Noncapsulated, i.e. nontypable (nt), strains are well-recognized causes of sinusitis and otitis media.\(^6\) They are also capable of causing invasive diseases such as bacteremia,\(^7\) pneumonia\(^8\) and meningitis.\(^9\) They are particularly recognized as a cause of neonatal sepsis.\(^10\), \(^11\) Serotype f (Hif) is reported as the most common cause of invasive encapsulated non-b Hi disease in children.\(^12\) Serotype a is a rare cause of pneumonia and meningitis\(^13\) and there are even less frequent reports of invasive disease in children caused by Hi serotype c (Hic),\(^14\) Hi serotype d\(^15\) and Hi serotype e (Hie).\(^16\), \(^17\)

Since October 1, 1992, Hib conjugate vaccines have been used routinely in the UK and the Republic of Ireland (ROI). After the introduction of routine Hib vaccination, we initiated a prospective national study to detect cases of invasive Hi disease in vaccinated and unvaccinated children. This allowed us to assess the clinical and epidemiologic factors associated with non-type b _H. influenzae_ disease in all British and Irish children and in vaccinated children to compare the features of non-type b disease cases with those of type b disease cases.

METHODS

This study was performed under the auspices of the British Paediatric Surveillance Unit (BPSU) of the Royal College of Paediatrics and Child Health. The BPSU has a program of active surveillance for selected rare pediatric conditions. Every month \(>90\%\) of pediatricians routinely report to the BPSU using the report card sent to them on a monthly basis.\(^18\)

Microbiologists and consultants in communicable disease control were also informed about the study and contributed to surveillance. In the ROI there was also active laboratory surveillance in which the investigator telephoned all pediatric laboratories every 2 weeks.

Pediatricians were asked to provide early notification by telephone. They were sent a questionnaire requesting clinical, demographic and laboratory information.

For the first phase of the study (October 1, 1992 to October 31, 1995), reports were requested of any child with invasive Hi disease who had received Hib conjugate vaccine. From November 1, 1995, the case definition was broadened to include all children with invasive Hi disease regardless of vaccination status. Invasive disease was defined as isolation of Hi from a normally sterile site (such as blood, cerebrospinal fluid, joint aspirate) or a positive Hib antigen test (in cerebrospinal fluid) combined with a clinical picture compatible with meningitis. The diagnosis of pneumonia required the presence of radiologic changes together with positive blood cultures for Hi. In the case of non-type b isolates the case was not included if other organisms were isolated at the same time.

The dates of all primary immunizations were obtained from the child’s general practitioner or from the district child health immunization computer records.

The local microbiologist was asked to send the isolate to the Public Health Laboratory Service _Haemophilus_ Reference Laboratory in Oxford where the identity was verified by standard serotyping and PCR techniques.\(^19\) In the ROI isolates were sent to Oxford via the microbiology department at the Waterford General Hospital.

To encourage maximum reporting of cases, the study was widely covered in the medical press before its commencement, and pediatricians were then updated through quarterly BPSU bulletins and an annual BPSU report.\(^18\) Updates were also posted in the Communicable Disease Weekly Report for England and Wales and the Weekly Report of the Scottish Centre for Infection and Environmental Health. Reports of Hi in children made to other agencies were checked against...
those reported to the study including the two pharmaceutical companies supplying the vaccines, the UK Department of Health Medicines Control Agency, the Public Health Laboratory Service Communicable Disease Surveillance Centre for England and Wales and the Scottish Centre for Infection and Environmental Health.

We compared the clinical features of type b and non-type b disease in fully vaccinated children. A case of Hi disease in a fully vaccinated child was defined as invasive disease occurring >1 week after two or more doses of Hib conjugate vaccine had been given to a child in the first year of life.

Statistical analysis was performed using SPSS (SPSS, Inc., Chicago, IL) and EPI-Info Version 6 (CDC, Atlanta, GA). Ages are given as medians (range) and compared with the Mann-Whitney test. Proportions are compared using the chi square test or Fisher’s exact test where an expected number was <5. No adjustments were made for multiple comparisons. Population and birth figures were obtained from the Office for National Statistics. During the period of the study the annual UK birth cohort varied between 717 000 and 781 000.

This study was approved by the Central Oxford Research Ethics Committee.

RESULTS

During the 6 years and 3 months between October 1, 1992, and December 31, 1998, there were 147 cases of invasive non-type b Hi disease reported in vaccinated children. During the 3 years and 2 months between November 1, 1995, and December 31, 1998, there were 84 cases of invasive non-type b Hi disease reported in unvaccinated children.

Hi disease in vaccinated children. Of the 147 non-b isolates in vaccinated children, nt strains accounted for 78% (115), Hif for 16% (23), Hie for 5% (7) and there was 1 each of Hi serotype a and Hi serotype c. The median age of presentation for all non-b cases was 16.8 months (2.4 to 95.2 months), and the most common modes of presentation were bacteremia (34%), meningitis (33%) and pneumonia (20%). There were no cases of epiglottitis.

During the 6 years between October 1, 1992, and October 1, 1998, there were 106 cases of invasive type b disease in fully vaccinated children (i.e. vaccine failures). Table 1 compares age, mode of presentation, presence of associated medical conditions and mortality in these children with children who were also fully vaccinated but had invasive non-b Hi disease during this same period (102 cases). Non-b cases are further divided into those with nt and those with non-b capsulate strains. The major differences include age at disease presentation (younger in those with non-b disease, especially non-b capsulate disease) and clinical presentation (epiglottitis only found and meningitis more common and bacteremia and pneumonia less common among Hib cases). Associated medical conditions were also more common among cases of nt Hi disease as compared with cases of type b disease (32% vs. 20%, P = 0.08).

The numbers of cases of type b and non-type b Hi in fully vaccinated children varied during the 6-year period. During the first 24 months the numbers of cases were similar (22 non-b vs. 18 Hib), whereas for the last 24-month period there were more cases of non-b Hi than Hib (58 non-b Hib vs. 38 Hib).

Hi disease in all children (vaccinated and unvaccinated). For the second period of the study (November 1, 1995, to December 31, 1998; 38 months) notifications of Hi disease were made for all children, regardless of vaccination status. Of a total of 188 non-b cases, 104 were vaccinated (included in analysis above) and 84 were not vaccinated. The majority were nt strains (165, 88%) followed by Hif (19, 10%), Hie (3) and Hic (1). The major modes of presentation were bacteremia (52%), meningitis (22%) and pneumonia (18%). The median age of presentation for non-b disease was

| TABLE 1. Comparison of age, clinical presentation, associated medical conditions and mortality between fully vaccinated children with type b or non-b Haemophilus influenzae |
|---|---|---|---|---|---|---|---|
| | Age Contracted Disease (mo) | Median | Range | No. of Cases |
|---|---|---|---|---|---|---|
| Hib | 22.1 | 3.8–64.0 | 65 (61)* | 20 (19) | 5 (5) | 6 (6) | 4 (4) | 5 (5) | 21 (20) | 1 (0.9) | 106 |
| Non-b Hib | 15.7† | 2.9–70.3 | 35 (34) | 0† | 30 (29) | 22 (22) | 1 (1) | 5 (5) | 29 (29) | 4 (3.9) | 102 |
| Hib | 19.6 | 2.9–70.3 | 23 (20) | 0† | 24 (30) | 21 (27) | 0 | 4 (5) | 25 (32)§ | 4 (5.1) | 79 |
| Non-b Hib (capsulated) | 8.9† | 5.4–64.5 | 12 (52) | 0§ | 6 (39) | 1 (4) | 1 (4) | 4 (17) | 0 | 23 |
| * Numbers in parentheses, percent. |
| † P = 0.08 vs. Hib. |
| ‡ P = 0.001 vs. Hib. |
| § P = 0.02 vs. Hib. |
| ¶ P = 0.004 vs. Hib. |
Onset disease 73% (30) were born prematurely (77% (37) in the first 2 days of life. Of those with early neonatal period (37), meningitis (30, 18%) and cellulitis (7, 4%). Forty-one percent had an associated medical condition.

**Nontypable H. influenzae disease.** The median age of nt Hi disease was 9.2 months (0 to 167.1) The major mode of presentation was bacteremia (89, 54%), followed by pneumonia (32, 19%), meningitis (30, 18%) and cellulitis (7, 4%). Forty-one percent had an associated medical condition.

Twenty-nine percent (48) of nt cases occurred in the neonatal period (≤30 days of age). Of the 48 cases 85% (41) occurred at <7 days of age (early onset disease), 77% (37) in the first 2 days of life. Of those with early onset disease 73% (30) were born prematurely (<37 weeks), 95% (39) presented with septicemia (2 with meningitis) and 17% (7) died (all were premature). The annual incidence of early onset neonatal nt disease in the UK is shown in Table 3.

**H. influenzae type f disease.** Hif presented at a median age of 16.8 months (5.4 to 154.4), and the majority of cases had meningitis (11, 58%) followed by bacteremia (5, 26%), pneumonia (2) and cellulitis (1). Five had associated medical conditions.

**H. influenzae type e disease.** The three cases of He disease presented with bacteremia at 2.9 and 54 months of age and peritonitis at 47 months of age, respectively. All had associated medical conditions.

**H. influenzae type c disease.** The one case of Hc developed bacteremia at 12 months of age. This child also had diabetes insipidus.

There were two strains that phenotypically appeared to be nontypable but genotypically were type b (b strains). One was obtained from a fully vaccinated child and the other from a partially vaccinated child.

**DISCUSSION**

We report the results of 6 years of surveillance of non-type b H. influenzae disease in children who have received the Hib conjugate vaccine and 3 years of surveillance of non-type b H. influenzae disease in all children (regardless of vaccination status) in the UK and ROI. This surveillance has been national, active and prospective, and a number of steps were taken to ensure that ascertainment was as complete as possible.

The Hib vaccine program has been well-accepted by children and parents in the UK and ROI. In the UK the impact on invasive Hib disease has been impressive, with a 98% fall in cases in children <5 years of age. Accurate Hib vaccine coverage figures are known, and >90% of UK children have received a full course of Hib vaccination by their fifth birthday (3 doses ≤13 months of age or 1 dose 13 to 48 months of age). For example 94% of children who were born at around the midpoint of this surveillance period (born April through June, 1995) have been fully vaccinated.

We estimate an annual incidence of non-b disease in children <5 years of age (shown in Table 3) which is ~30-fold lower than that of Hib disease in the prevaccine era and in accord with that calculated in previous UK and US studies. Non-b Hi is therefore a rare cause of disease. However, in 1998 the incidence of non-b Hi disease was twice that of Hib disease in children <5 years of age and three times the incidence of Hib disease in children <1 year of age (Table 3). Furthermore in fully vaccinated children Hi disease is now more likely to be caused by non-b Hi than by Hib. In the absence of full identification of the strain causing disease, this might conceivably result in a significant underestimation of vaccine effectiveness; in this study at least twice as many vaccine failures would have been reported if the strain causing disease had not been fully verified.

Comparison of type b and non type-b cases of Hi reveals a striking difference in clinical presentation; Hib presents predominantly with meningitis and epiglottitis and infrequently with pneumonia and bacteremia, whereas non-b Hi presents less commonly with meningitis and much more commonly with bacteremia and pneumonia. When non-b disease is further divided into encapsulated and nonencapsulated strains, it becomes apparent that capsulated strains (predominantly serotype f) occur in younger children with a predominance of meningitis and bacteremia whereas nonencapsulated (nontypable) strains account for the majority of pneumonia among non-b cases. Epiglottitis appears to be overwhelmingly a syndrome associated with serotype b.

Consistent with other population-based studies in the UK, US the Netherlands and Switzerland nontypable Hi accounts for most non-b Hi disease. One exception to this is a US report of Hi
disease in 1994 and 1995 which appeared to show that non-b capsulated strains were a more frequent cause of invasive disease than nontypable strains in children <5 years of age.\(^{15}\) However, only 56% of isolates in this age group were serotyped; therefore the reported distribution of serotypes may not be accurate.

Underlying or associated medical conditions appear to be frequent in children with non-b Hi disease. In contrast such conditions were uncommon among cases of Hib disease in the prevaccine era\(^ {25}\) and reinforce the difference in virulence between non-b and type b Hi.

It must be emphasized, however, that non-b cases are capable of causing significant disease. Of a total of 208 cases of non-b Hi in this study, 58 (25%) presented with meningitis and the mortality of non-b cases was \(\approx 9\%\), twice the mortality of type b disease in the prevaccine era.\(^ {3}\)

Urwin et al.\(^ {12}\) reported 19 cases of Hif in US children in a 6-year period. We describe a total of 28 children, 9 from the period in which only vaccinees were eligible for inclusion and 19 from the 38-month period in which all childhood cases were sought. In keeping with Urwin et al.,\(^ {12}\) of those children <5 years of age, the majority were <12 months of age. Meningitis and pneumonia accounted for 40% of disease respectively in the US children, whereas in the UK children there was a slightly greater proportion of meningitis (58%) and more bacteremia (26%). We had no deaths as compared with 4 of 19 deaths in the US study and there were similar proportions with underlying conditions (26%). These data add to and are compatible with that of Nitta et al.\(^ {26}\) who described 3 cases of Hif and reviewed another 12 cases from the literature and also found a predominance of meningitis, underlying abnormalities (in 1 of 3) and no deaths.

It may be concluded from this and other reports that invasive disease in our population caused by serotypes a (one case), c (one case) and d (no cases) is extremely rare. Hia as a cause of meningitis and pneumonia has been reported more frequently in certain native populations.\(^ {27, 28}\)

Noncapsulate (nontypable) Hi is recognized as an uncommon pathogen in the neonatal period.\(^ {11, 29–31}\) Quentin et al. reported an incidence of disease of 2.8/100 000 live births, but others have reported rates of 4.6/100 000 live births,\(^ {11}\) 2.8/100 000\(^ {31}\) and 1.6 to 1.9/100 000 in this report. The predominance of prematurity and the onset of disease soon after birth are features common to all reports. However, we, like Falla et al. and Takala et al., did not find a preponderance of biotype 4 as has been described by others (Refs.\(^ {29}\) and \(^ {30}\) and data not shown). To put this in perspective the rate of early onset neonatal nontypable disease determined in this study is one-fortieth that of early onset group B streptococcal disease in the UK.\(^ {32}\)

Two strains were genotypically b−. If identification of isolates relied solely on conventional capsular serotyping, the presence of such strains could result in an overestimation of Hib vaccine efficacy. They may also represent a theoretical means by which Hib could evade the effect of Hib vaccine. There is no way of knowing, however, whether these particular isolates were expressing capsule at the time of invasion.

We conclude that non-type b Hi is a rare cause of disease in children. With the decline of Hib, however, it is a relatively more important pathogen and is now the most common strain of Hi isolated from children who have received Hib conjugate vaccine. Care must therefore be taken in verifying the isolate in cases of possible vaccine failure. This group of organisms is capable of causing severe diseases such as meningitis; their most common modes of presentation are with bacteremia and pneumonia. They also may signal an underlying host abnormality and we would recommend that underlying conditions should be sought.

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REFERENCES


