THE SEARCH FOR ADENOVIRUS 14 IN CHILDREN IN HOUSTON, TEXAS

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Abstract: Adenovirus (Ad)14 has recently emerged in the United States causing outbreaks of severe respiratory disease. To determine if Ad14 circulated in Houston, Texas, during the same time as an outbreak in military recruits in nearby San Antonio, 215 pediatric adenovirus isolates were serotyped using microneutralization. None were Ad14; Ad1, Ad2, and Ad3 were the most common identified serotypes.

Key Words: adenovirus 14, serotypes, children, epidemiology

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Adenoviruses (Ads) are responsible for a varied array of clinical syndromes in children and adults.[1,2] Of the 51 known Ads serotypes affecting humans, Ad14 has been increasingly detected in the United States since March of 2006,[3] simultaneously emerging at 5 of 8 U.S. military training facilities participating in a population-based acute respiratory surveillance.[4] At Lackland Air Force Base in San Antonio, Texas, 63% of 423 specimens obtained from February to June 2007 from military recruits with febrile respiratory illness detected adenovirus, of which 90% were typed as Ad14.[3] A recent epidemiologic study also detected Ad14 in military recruits, raising concern that Ad14 may replace other group B serotypes such as Ad3, Ad7, and Ad21 as a cause of illness in new trainees.[5] Adenovirus 1, Ad2, and Ad3 seem to predominate in civilian adult and pediatric populations, with Ad14 rarely, if ever detected.[5]

Like other group B adenoviruses, Ad14 may have the potential to cause major community outbreaks in children. We hypothesized that the Ad14 outbreak at Lackland Air Force Base in San Antonio, located approximately 180 miles west of Houston, should disperse rapidly into the Ad14 naive pediatric community in Houston. To test our hypothesis we retrospectively performed serotyping analysis in all adenovirus positive viral culture specimens received between January 1, 2007 and June 30, 2007 in the Diagnostic Virology Laboratory at Texas Children's Hospital (TCH). This period overlapped with the abovementioned detection of Ad14 in San Antonio, TX.

PATIENTS AND METHODS

Patients, Specimens, and Cultures. Clinical specimens (respiratory, urine, or fecal sources) from patients with viral syndromes were inoculated onto HFF (human foreskin fibroblast), RhMK (rhesus monkey kidney cells), and A549 (human alveolar basal epithelial carcinoma) monolayers and observed for viral cytopathic effect for a maximum of 14 (for RhMK and A549) and 21 (for HFF) days. Virus identification was confirmed using virus specific immunofluorescence assay (Diagnostic Hybrids, Athens, OH), performed according to the manufacturer's instructions.

Serotyping. Because prior data showed that Ad1, Ad2, and Ad3 causes most pediatric infections,[6] Ad positive specimens were serotyped by microneutralization assay, with reference antisera (ATCC, Rockville, MD) against Ad1, Ad2, Ad3 and the serotype of interest Ad14, as previously described.[6] Reference adenoviruses (ATCC) were used as positive controls.

RESULTS

From January 1 to June 30, 2007, 4962 specimens were submitted for viral cultures to the Diagnostic Virology Laboratory at TCH. Of these, 215 (4.3%) were positive for adenovirus. Two hundred and five (95%) specimens were available for serotyping. These specimens corresponded to 174 patients, with 22 children having 2 or more positive specimens. Patients' mean age was 4.25 years (range, 25 days–26 years). Most of the specimens were obtained from children treated at the emergency department (n = 119, 58%), the intensive care units (n = 15; 7%), and the bone marrow transplant unit (n = 14; 7%). Samples were of respiratory, fecal, or other source in 83%, 16%, and 1%, respectively. Adenovirus 3 was the most common Ad type and no specimen was identified as Ad14 (Table 1). Forty-one samples grew adenoviruses that were not identified with antisera to Ad1, Ad2, Ad3, or Ad14. Twenty-five samples could not be reisolated in 293 cells for serotyping.

DISCUSSION

Despite the appearance of clusters of Ad14 in military recruits from a geographic area in close proximity, as well as in several other states, Ad14 was not detected in our pediatric population. Adenoviruses 1, Ad2, and Ad3 were the major serotypes detected in children treated at TCH, consistent with prior epidemiologic studies.[5,6] Adenovirus surveillance contributes to the understanding of the evolving epidemiology of adenovirus serotypes and their different clinical manifestations. We had hypothesized that Ad14 once introduced into the community should result in explosive outbreaks similar to Ad3 and Ad7, which are also group B adenoviruses. Because a community outbreak with Ad14 has not occurred in Houston thus far, other epidemiologic factors required for widespread dissemination may not be present in the community.[5,6]

Significant outbreaks with Ad4, Ad7, Ad21, and now Ad14 at military training centers with attack rates of 40–90% can occur within the first 8 weeks of basic training,[1] but outbreaks of severe adenovirus disease in children in the United States, so far, are not common. Of the clusters of patients from the states of Oregon, Washington, and Texas, the Centers for Disease Control and Prevention reported that in all instances the Ad14 sequence was distinct from the reference strain from 1955, suggesting viral mutation that enhanced virulence, although it conserved the neutralizing epitopes.[3] It is therefore possible an Ad14 outbreak may occur in the future, and include infants in day care centers, immunocompromised patients, and college students in dormitories, all of whom may be in daily proximity to each other, similar to military recruits. On the other hand, the high prevalence of Ad3 in the pediatric population may provide cross-reactive cell-mediated immunity to Ad14 through conserved cytotoxic T lymphocyte epitopes on the adenovirus hexon protein sufficient to reduce the likelihood of an Ad14 outbreak in the pediatric community.[7]

Several limitations exist in this study. The microneutralization method for serotyping adenoviruses may not adequately discriminate coinfection with multiple serotypes. Furthermore, a minority of adenovirus-positive samples could not be reclaimed for serotyping, so it is possible some of these specimens contained Ad14. Also, in part because of the retrospective nature of this study, mild cases of Ad14 could have been missed (incomplete capture) because samples were obtained at the discretion of the physicians,

TABLE 1. Serotype Distribution of Adenoviruses Isolated From Pediatric Patients From January to June 2007, in Houston, Texas

Serotype	n	%
Adenovirus 3	97	54
Adenovirus 2	27	15
Adenovirus 1	15	8
Adenovirus 14	0	0
Adenovirus not typed*	41	23
Total†	180	100

*Adenovirus isolates not typed as Ad1, Ad2, Ad3, or Ad14.
†Twenty-five samples could not be reisolated for serotyping.

and some patients may not have had viral cultures performed. Finally, because this study encompassed only 6 months, it is possible the serotype distribution may show seasonal variation and longer periods of seroepidemiologic observation may be required to confirm these preliminary findings.

In conclusion, despite the emergence of severe disease as a result of Ad14 in other populations, during a 6-month observation period Ad14 infection was not detected, and confirmed the current predominance of adenovirus types 1, 2 and 3 in pediatric patients in Houston, TX. We hypothesize that the high prevalence of Ad3 in our community is providing cross-protection against Ad14.

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A SCORE IDENTIFYING SERIOUS BACTERIAL INFECTIONS IN CHILDREN WITH FEVER WITHOUT SOURCE

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Abstract: The objective of the study was to develop a simple clinical tool to identify serious bacterial infection (SBI) in children with fever without a source. For each child, a clinical assessment, a white blood cell count, a urine analysis, a determination of C-reactive protein, procalcitonin, and appropriate cultures were performed. Two hundred two children were studied of whom 54 (27%) had SBI. In the multivariate analysis, only procalcitonin [odds ratio (OR): 37.6], C-reactive protein (OR: 7.8), and urine dipstick (OR: 23.2) remained significantly associated with SBI. The sensitivity of the score for the identification of SBI was 94% and the specificity 81%. In the validation set the sensitivity of the score was 94% and the specificity 78%.

Key Words: scoring system, fever without a source, serious bacterial infection

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Fever without source (FWS) in young children remains a difficult diagnostic problem, because clinical signs and symptoms are often unreliable predictors of a serious bacterial infection (SBI). Many clinical studies have addressed this problem, and the combination of a clinical evaluation associated with a total and differential leukocyte count are commonly used screening methods.[1–3] The relatively poor specificity of the markers used to identify SBI, taken independently, urges physicians to give antibiotics to the majority of patients. In our study, we analyzed the predictive values of different markers in a multivariate logistic regression analysis. Our goal was to develop a simple score, which could be easily performed in the emergency room or in the office to predict SBI in a pediatric population with FWS.

MATERIALS AND METHODS

We performed a combined analysis of data collected from 2 prospectively and consecutively enrolled cohorts of children with FWS in a single university center.[4,5] Both cohort studies had the same inclusion and exclusion criteria and had followed similar methodology. The study protocol was approved by the Ethical Committee of the Child and Adolescent Department, University Hospitals of Geneva. The study included all children aged from 7 days to 36 months who were consecutively admitted to the Emergency Department of the University Children's Hospital of Geneva with a rectal temperature above 38°C and without localizing signs of infection in their history or at physical examination. Criteria of exclusion are notified in the previous studies.[4,5] All children had a clinical score based on the Infant Observation Scale (IOS),[6] a urine analysis with culture and blood drawn for white cell count, determination of C-reactive protein (CRP), procalcitonin (PCT), and culture. Lumbar puncture was performed when meningitis was suspected. The pediatric resident in charge of the patient decided which child should receive antibiotics. All children had a clinical follow-up with physical examination by a pediatrician in the following 48 hours or by a telephone contact. The diagnosis was registered at the end of the clinical follow-up. Technical laboratory determinations and definition of SBIs: bacteremia, pyelonephritis, lobar pneumonia, bacterial meningitis, and criteria of benign infection are described elsewhere.[4]

Statistics. The study population was divided by stratified randomization in a derivation set (2/3) and a validation set (1/3). The sensitivity, specificity, negative, and positive predictive values for the detection of a SBI were determined in the derivation set for the different laboratory parameters using the cutoff points derived from our previous studies.[4,5] Univariate logistic regression was performed considering the dichotomized predictive parameters as independent values and SBI as the outcome value. Then, parameters significantly

associated with SBI were entered forward stepwise into a multiple regression model and only those remaining independently significantly ($P < 0.05$) associated with SBI were retained. For ease of use in the clinical setting, we then created a Laboratory-score using only the predictive variables independently associated with SBI. The sensitivity, specificity, and predictive values of the Laboratory-score were determined in the derivation set and in the validation set.

RESULTS

Two hundred twenty-two children were consecutively included from March 1998 to February 2002. Twenty children were excluded. The data of 202 children were analyzed. The final diagnosis was: SBI in 54 children (27%) (7 bacteremia, 40 pyelonephritis, 5 lobar pulmonary condensation, 1 retropharyngeal abscess, and 1 mastoiditis), benign focal infection in 26 children (13%) (cystitis, acute otitis media, adenitis, Campylobacter gastroenteritis), and probable viral infection in 122 children (60%) (negative culture and no signs for focal infection at clinical follow-up). One hundred thirty-four of 202 (66%) of the children received antibiotics. The study population was divided in a derivation set (n = 135) and a validation set (n = 67). The 2 sets were comparable in terms of age, fever, incidence of SBI, clinical observational scores, and laboratory parameters. The sensitivity, specificity, and predictive values for the different parameters associated with SBI are listed in Table 1.

Logistic Regression. We first performed univariate logistic regression with variables potentially associated with SBI. PCT [odds ratio (OR): 35.6] showed the strongest association followed by CRP (OR: 12.9), urine dipstick (OR: 9), and leucocytosis (OR: 3). Left shift and IOS score were not statistically associated with SBI.

Then PCT, CRP, urine dipstick, and leucocytosis were entered into a forward stepwise multiple logistic regression model to identify independent predictor of SBI. The PCT value remained the most significant predictor of SBI (OR: 37.6; 95% CI: 5.8–243). The other variables independently associated with SBI in this analysis were CRP (OR: 7.8; 95% CI: 2–30.4) and urine dipstick (OR: 23.2; 95% CI: 5.1–104.8). Leucocytosis was not independently associated with the occurrence of SBI ($P = 0.49$).

Laboratory-Score. Based on the results of the logistic regression analysis, we developed a risk index score, named Laboratory-score. The relative weighting of each component variable of the Laboratory-score was based on its odds ratio in the univariate analysis. Two points were attributed to PCT or CRP above the cutoff values (0.5 ng/mL and 40 mg/L, respectively) and 4 points for values of PCT above 2 ng/mL, and for CRP above 100 mg/L. One point was attributed for a positive urine dipstick (Table 2).

The performance of the Laboratory-score was then tested both on the derivation population and the validation set (Table 1). In the derivation set, the Laboratory-score (≥3) had a sensitivity of 94% and a specificity of 81%. When compared with the other parameters commonly used to predict SBI, the Laboratory-score had the best accuracy associating good sensitivity and specificity. In the validation set the Laboratory-score had similar performances with a sensitivity of 94% (95% CI: 74–99) and a specificity of 78% (95% CI: 64–87) (Table 1).

TABLE 2. Laboratory Score

Predictor	Points
PCT (ng/mL)	
<0.5	0
≥0.5	2
≥2	4
CRP (mg/L)	
<40	0
40–99	2
≥100	4
Urine dipstick*	
Negative	0
Positive	1

Possible laboratory scores range from 0 to 9.
*Positive urine dipstick: positive leukocytes esterase, or nitrite test result.

DISCUSSION

Our data showed that PCT, CRP, and urine dipstick are independent predictors of SBI in this population of children with FWS. In our study, the IOS score and left shift were not statistically different between children with and without SBI. Moreover, leucocytosis was not an independent predictor of SBI when PCT, CRP, and urine dipstick have been taken into account.

We have developed a scoring system (Laboratory-score) based on the 3 predictive variables independently associated with SBI: PCT, CRP, and urinary dipstick. The principal advantage of the Laboratory-score is its good specificity (81%) for the prediction of SBI associated with the security of a high sensitivity (94%). The good specificity of the Laboratory-score should enable the reliable selection of children who need antibiotic treatment, without over treating children with viral infection. Based on this study, if antibiotics had solely been administrated for children with a positive score, only 40% of the population would have received antibiotics. In comparison, based on the clinician's decisions, more than 65% of the studied population received antibiotics. The use of the Laboratory-score could, thus, substantially reduce antibiotic use.

Potential limitations of our study should be considered. Our study population is relatively small explaining the wideness of the confidence intervals around the estimates of sensitivity and specificity. The incidence of SBI (27%) in our study seems higher than reported in other studies,[7–9] but similar to the incidence of a recent study from Italy (23%) that analyzed comparable populations of children in a tertiary hospital.[10] This likely reflects referral bias, as pediatricians refer ill-appearing children to our hospital for initial work-up. Because this bias

TABLE 1. Predictive Value (%) of Different Variables Between Children With and Without Severe Bacterial Infections

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
PCT*(0.5 ng/mL)	94 (82–99)	68 (58–76)	51 (40–63)	97 (90–99)
CRP*(40 mg/L)	81 (65–90)	76 (67–83)	55 (42–67)	92 (83–96)
Positive urine dipstick	67 (50–80)	82 (73–89)	57 (42–71)	87 (79–93)
Leucocytosis*(15 G/L)	53 (37–69)	73 (63–81)	41 (28–56)	81 (72–88)
Left shift*(1.5 G/L)	17 (8–32)	91 (84–95)	40 (20–64)	75 (67–82)
Laboratory score*(3)				
Derivation pop (n = 135)	94 (82–99)	81 (72–88)	64 (51–76)	98 (92–99)
Validation pop (n = 67)	94 (74–99)	78 (64–87)	61 (42–76)	97 (87–100)

*Cutoff level.
PPV indicates positive predictive value; NPV, negative predictive value.

affects the prevalence of SBI in our patient population, the predictive values of the Laboratory-score must be interpreted with caution, and the performance of the Laboratory-score might vary if applied to other cohorts of children. In contrast, the sensitivity and specificity of our scoring system are not affected by this potential bias. An internal validation of the score was performed on a subset of the population. However, this sample is small and the potential bias associated with our entire population remains.

In conclusion, PCT, CRP, and urine dipstick are independent predictors of SBI in this study. White blood cell count is not an independent predictor, when these 3 variables are taken into account. A Laboratory-score including PCT, CRP, and urine dipstick provides a security equivalent to the standard work-up, is easier to use, and could considerably diminish antibiotic use in children with benign infection. However, children should be carefully followed up, to identify the small proportion with SBI not initially detected by a positive score. Finally, the Laboratory-score should be prospectively validated and evaluated in different clinical settings before its use in clinical guidelines of children with FWS.

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EFFICACY OF PENTAVALENT HUMAN-BOVINE (WC3) REASSORTANT ROTAVIRUS VACCINE BASED ON BREASTFEEDING FREQUENCY

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Abstract: The efficacy of a live, oral, pentavalent rotavirus vaccine against G1-4 rotavirus gastroenteritis (RVGE) was retrospectively assessed based on breastfeeding frequency among 5098 infants in a placebo-controlled trial. The efficacy against any RVGE severity for infants never breastfed, sometimes breastfed, or exclusively breastfed was 68.3%, 82.2%, and 68.0%, respectively. The efficacy against severe RVGE was 100%, 95.4%, and 100%, respectively. Breastfeeding did not seem to adversely impact the efficacy of pentavalent rotavirus vaccine.

Key Words: rotavirus, gastroenteritis, pentavalent rotavirus vaccine, breastfeeding

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Rotavirus is the leading cause of severe dehydrating gastroenteritis in infants and young children worldwide. Most of the ~611,000 rotavirus-attributable deaths each year occur in resource-limited regions of the world.[1,2] Improving water supply and sanitation has had little impact on the ubiquitous nature of rotavirus disease, as nearly all children worldwide are infected by 5 years of age. Thus, vaccination seems to be the most effective strategy to prevent rotavirus disease in both the developing and developed world.

Successful implementation of a safe and effective rotavirus vaccine into routine immunization schedules is expected to reduce greatly the global burden of rotavirus disease, but requires that the vaccine fit into existing health practices. Breastfeeding is a common practice in the developed world and is virtually universal in the developing world because it provides an ideal source of infant nutrition. Transfer of maternal antibody to infants through human milk confers protection against morbidity and mortality from bacterial, viral, and parasitic infections.[3] Early and exclusive breastfeeding has been shown to reduce the risk of postnatal HIV-1 transmission relative to intermittent breastfeeding.[4] Accordingly, government and nongovernment organizations continue to strongly promote breastfeeding in most underprivileged populations.[5] Because vaccination seems to be the most effective strategy to prevent rotavirus disease in both the developed and developing world, it is important to understand the impact of breastfeeding on vaccine efficacy.

A pentavalent rotavirus vaccine (PRV), RotaTeq (Merck & Co., Inc, Whitehouse Station, NJ), was licensed in the United States in February 2006, and has now been licensed as of May 2008 in 85 countries. The vaccine contains 5 live human-bovine reassortant rotaviruses, each consisting of the WC3 bovine strain with viral surface proteins corresponding to human rotavirus serotypes G1, G2, G3, G4, and P1A[8]. The Rotavirus Efficacy and Safety Trial (REST) demonstrated that after 3 doses, vaccine efficacy was 74% against G1-4 rotavirus gastroenteritis (RVGE) of any severity and 98% against severe G1-4 RVGE compared with placebo. The vaccine also decreased the rate of healthcare resource utilization for RVGE, including a 95% reduction in hospitalizations.[6]

Rotavirus vaccine is targeted for use starting at 6 weeks of age to prevent the majority of RVGE that begins to peak at ~6 months. Protection against severe disease of younger infants may be mediated by passively transmitted maternal antibodies. Vaccination in early infancy may also be impacted by maternal antibodies. The presence of relatively high concentrations of maternal IgG may inhibit rotavirus infection in the infant gut.[7] Transfer of antibodies via breast milk is thought to play a role. In addition, rotavirus IgA has been detected in stools of breastfed, but not bottlefed, neonates.[8]

Thus, it is important to assess whether breastfeeding, with the potential transfer of maternal rotavirus antibodies, affects the ability of the vaccine to protect against RVGE, especially because it is a live, oral vaccine administered in infancy. The large REST database

TABLE 1. Efficacy Against Any and Severe G1-4 Rotavirus Gastroenteritis Through the First Full Rotavirus Season Based on Frequency of Breastfeeding (Per Protocol Population and Analysis)

Breastfeeding Frequency	Any G1-4 Rotavirus Gastroenteritis			Severe G1-4 Rotavirus Gastroenteritis		
	No. Cases/Number Vaccinated		Efficacy (95% CI)	No. Cases/Number Vaccinated		Efficacy (95% CI)
	Vaccine Recipients	Placebo Recipients		Vaccine Recipients	Placebo Recipients	
Never	19/815	60/817	68.3% (46.1–82.1)	0/815	9/817	100% (48.3–100)
Some	24/953	133/947	82.2% (72.3–89.0)	1/953	22/947	95.4% (71.3–99.9)
Exclusively	39/767	122/799	68.0% (53.8–78.3)	0/767	20/799	100% (79.3–100)

provided a unique opportunity to assess the impact of breastfeeding practices on vaccine efficacy. In this report, we describe the efficacy of PRV in infants according to breastfeeding frequency.

METHODS

REST was a large-scale, blinded, placebo-controlled, randomized trial conducted in 11 countries. The study was designed to assess the safety (primarily with respect to intussusception) and efficacy of PRV. A substudy, nested within REST, evaluated the efficacy of the vaccine against all episodes of RVGE.

Details of the study have been previously published.[6] In brief, healthy infants between 6 and 12 weeks chronologic age were eligible. Concomitant administration of other licensed childhood vaccines and breastfeeding were permitted during the study. Study participants were randomized 1:1 to receive 3 oral doses of PRV or visibly indistinguishable placebo. Each dose was administered ~4 to 10 weeks apart. In the clinical efficacy substudy, parents/guardians were to report all episodes of acute gastroenteritis in infants occurring after the first dose through the next full rotavirus season. Active surveillance for RVGE, including biweekly contacts with the parent/guardian, was conducted during the rotavirus season.

A case of RVGE was defined as ≥3 watery or looser-than-normal stools within a 24-hour period and/or forceful vomiting, along with rotavirus antigen detection by enzyme immunoassay in a stool sample collected within 14 days of symptom onset. The primary analysis of efficacy was based on wild-type cases of RVGE caused by serotypes G1, G2, G3 or G4 confirmed by reverse transcription-polymerase chain reaction that occurred ≥14 days after the third dose through the first full rotavirus season postvaccination. A validated clinical scoring system based on the intensity and duration of fever, vomiting, diarrhea, and behavioral changes was used to rate the severity of the RVGE; a score >16 out of a possible 24 was designated as severe disease.

At the time of administration of each dose of vaccine/placebo, the parent/guardian was asked to categorize his/her infant's feeding into 1 of 5 categories ranging from "breastfeeding only" to "formula feeding only." Breastfeeding status was subsequently summarized using 3 categories—never breastfed, some breastfeeding (any of 3 categories involving both breast and formula feeding), or exclusively breastfed.

No formal efficacy hypotheses were prespecified based on the frequency of breastfeeding. The efficacy of the vaccine in preventing any G1-4 RVGE or severe G1-4 RVGE through the first full rotavirus season postvaccination according to breastfeeding frequency was examined observationally based on cases that met the prespecified definition of RVGE occurring at least 14 days after the third dose. Efficacy was calculated as in REST.[6]

RESULTS

Data regarding breastfeeding frequency were available for 5098 infants in the per-protocol population of the efficacy substudy of REST. Of these infants, 1632 (32%) were never breastfed, 1900 (37%) received some breastfeeding, and 1566 (31%) were exclusively breastfed. The demographic characteristics of the infants in each of these 3 breastfeeding groups were generally similar. The median age at study entry was ~10 weeks in all 3 groups.

The efficacy against G1-4 RVGE was generally comparable for infants who were never, sometimes, or exclusively breastfed. The efficacy estimates against disease of any severity were 68.3%, 82.2%, and 68.0%, respectively; the efficacy estimates against severe disease were 100%, 95.4%, and 100%, respectively (Table 1).

DISCUSSION

Information on the efficacy of vaccines administered to breastfed infants is often limited or not available from controlled clinical trials. Because REST was one of the largest vaccine trials ever conducted, a substantial number of breastfed infants were enrolled. The efficacy of PRV against G1-4 RVGE of any severity and against severe disease was generally comparable in the large number of children studied regardless of the frequency of breastfeeding. These findings support implementation of this live, oral rotavirus vaccine program in areas of the world where children are breastfed.

Studies conducted with the previously licensed, oral, live tetravalent rotavirus vaccine (RRV, Rotashield, Wyeth-Ayerst) yielded conflicting results as to whether breastfeeding might impair vaccine performance.[9,10] The efficacy results of our study with PRV are comparable to the results of a previous study with RRV where seroresponses and protection rates in breastfed and nonbreastfed American children were generally similar.[9] However, in a later meta-analysis of immunogenicity data, breastfeeding was shown to have an adverse effect on seroconversion to RRV.[10] Although some immune indices may have been affected by breastfeeding, the effectiveness of RRV in breastfed infants apparently was not. A clear immune correlate for protection against rotavirus disease has not been established.

Although definitive conclusions cannot be drawn from this post hoc analysis, these data suggest that PRV is efficacious in infants regardless of their breastfeeding status. Our results further imply that breastfeeding alone is unlikely to have a major negative effect on the efficacy of PRV in the developing world where breastfeeding is common practice. Clinical trials are underway in Africa and Asia to establish the safety and efficacy of this vaccine in resource-limited regions where breastfeeding is common practice.

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IMMUNOGENICITY AND SAFETY OF AN INACTIVATED HEPATITIS A VACCINE ADMINISTERED CONCOMITANTLY WITH A PNEUMOCOCCAL CONJUGATE VACCINE IN HEALTHY CHILDREN 15 MONTHS OF AGE

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Abstract: The immunogenicity and safety of hepatitis A vaccine and pneumococcal conjugate vaccine, administered separately or concomitantly in children 15 months of age, were evaluated. After completed vaccinations, antihepatitis A and antipneumococcal geometric mean concentrations were similar across groups. Both vaccines were well-tolerated when given concomitantly during the second year of life.

Key Words: hepatitis A, Havrix, childhood vaccination, HAV, Prevnar

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In the United States, childhood vaccination has played a role in the decline in reported hepatitis A cases (4488 in 2005 versus 10,616 in 2001),[1] yet infected asymptomatic children remain as a potential source of transmission.[2] The Advisory Committee on Immunization Practices now recommends hepatitis A vaccination of all children in the United States beginning at age 1 year (12–23 months).[3] This study evaluated safety and immune responses to Havrix, GlaxoSmithKline Biologicals' inactivated hepatitis A vaccine (HAV) and Prevnar, Wyeth Pharmaceuticals' pneumococcal conjugate vaccine (PCV), administered separately or coadministered to children 15 months of age.

MATERIALS AND METHODS

Participants

In this open, randomized, multicentered study, healthy children were assigned to 1 of 3 groups with study vaccines given at 15 months of age. All children received 3 priming doses of pneumococcal conjugate vaccine during their first year of life; none received hepatitis A vaccine. Group HAV received hepatitis A vaccine. Group HAV+PCV received the first dose of hepatitis A vaccine coadministered with pneumococcal conjugate vaccine. Group HAV→PCV received the pneumococcal vaccine followed by the first dose of hepatitis A vaccine 1 month later. In all groups, the second dose of hepatitis A vaccine was given 6–9 months after the first dose. In the HAV, HAV+PCV, and PCV→HAV groups, 2.5%, 1%, and 2.5% of subjects, respectively, were seropositive at the prevaccination time point.

Study Design

Subjects were enrolled between 12 and 14 months of age. Three blood samples were collected for each group. Blood samples were taken at baseline (day 0, prestudy vaccination) and 31 days after the second dose of hepatitis A vaccine, regardless of when the second dose was administered. Additional samples from the HAV and HAV+PCV groups were taken 1 month after the first dose of study vaccinations, either hepatitis A vaccine or hepatitis A vaccine coadministered with pneumococcal conjugate vaccine. In the PCV→HAV group, an additional sample was taken 30 days after the pneumococcal vaccine dose.

Vaccines

Vaccines were administered intramuscularly. One dose (0.5 mL) of hepatitis A vaccine contained 720 enzyme-linked immunosorbent assay units of inactivated hepatitis A viral antigen and aluminum hydroxide 0.25 mg. One dose (0.5 mL) of pneumococcal vaccine contained 2 mcg saccharide of each of the following serotypes: 4, 9V, 14, 18C, 19F, and 23F, and 4 μg of serotype 6B (16 μg of total saccharide), 20 μg of CRM_{197} carrier protein, and 0.125 mg of aluminum phosphate.

Objectives: Immunogenicity Statistical Analysis

The according-to-protocol (ATP) cohort was used for immunogenicity analysis. The coprimary objectives were to evaluate whether the antihepatitis A virus immune response (seropositivity rates and geometric mean concentrations), 31 days after the second dose of hepatitis A vaccine when the first dose of hepatitis A vaccine was coadministered with pneumococcal conjugate vaccine, was noninferior compared with hepatitis A vaccine administered alone. A secondary objective was to evaluate whether the immunogenicity, with respect to geometric mean concentration (GMC), of pneumococcal capsular polysaccharides 31 days after the coadministration of pneumococcal conjugate vaccine with the first dose of hepatitis A vaccine was noninferior compared with pneumococcal conjugate vaccine when administered alone.

Immune Response

Hepatitis A antibody responses were measured by enzyme-linked immunosorbent assay (ELISA). Seropositivity was defined as

an antibody concentration ≥15 mIU/mL (assay cut-off value). An ELISA was used to quantitate pneumococcal antibodies (cut-off value of 0.05 μg/mL).[3]

Safety Evaluation

Postvaccination reactogenicity was assessed for 3 days after each vaccination. Diary cards were used to record solicited local (injection site) and general symptoms, including fever and irritability/fussiness, drowsiness, and loss of appetite. Serious adverse events (AEs) were reported throughout the duration of the study.

RESULTS

Participants

Five hundred twenty-one subjects were enrolled (177, 169, and 175 in the HAV, HAV+PCV, and PCV→HAV groups, respectively); 88 subjects withdrew before first vaccination. The ATP cohort included 123, 107, and 125 subjects in the HAV, HAV+PCV, and PCV→HAV groups, respectively.

Immunogenicity

Antihepatitis A virus seropositivity and GMC values are presented in Table 1. Noninferiority of the antihepatitis A virus immune response with respect to GMC was demonstrated. After the second dose of hepatitis A vaccine, the lower limit (0.63) of the 95% CI on the antihepatitis A virus GMC ratio (the HAV+PCV group divided by the HAV group) was higher than the predefined noninferiority limit of 0.5.

A single child in the HAV+PCV group did not maintain seropositive status after the second dose of hepatitis A vaccine. The difference between the HAV and HAV+PCV groups in antihepatitis A virus seropositivity rates in the ATP cohort was −1.06% (95% CI: −5.78 to 2.45). This coprimary objective was not achieved, as the lower limit (−5.78%) was less than the predefined lower limit of the 95% CI of −5%.

Seropositivity and GMCs for the 7 pneumococcal serotypes were assessed. For the HAV+PCV and HAV→PCV groups, seropositivity for all pneumococcal serotypes ranged from 98.3% to 100% one month after pneumococcal vaccination. The postvaccination seropositivity rates of the 7 pneumococcal capsular polysaccharides after pneumococcal conjugate vaccine in the HAV+PCV and PCV→HAV groups were similar for each of the 7 pneumococcal serotypes. For each pneumococcal serotype, the GMC ratio (HAV+PCV divided by PCV→HAV) was greater than 0.5; noninferiority was demonstrated.

After the first dose of hepatitis A vaccine in the HAV and HAV+PCV groups, 84.0% and 88.3% of subjects were seropositive, respectively, regardless of prevaccination seropositivity status. GMCs for subjects in the HAV and HAV+PCV groups 1 month after the first dose of hepatitis A vaccine were 48.0 mIU/mL and 61.0 mIU/mL, respectively.

The antihepatitis A virus immune response, with respect to seropositivity rate and GMC, in the PCV→HAV group was assessed. After the second dose of hepatitis A vaccine, 98.3% of subjects were seropositive and the antihepatitis A antibody GMC was 1391.2 mIU/mL.

TABLE 1. Antihepatitis A Virus Seropositivity and GMCs ATP Cohort*

Group	Time	N	Seropositivity (≥15 mIU/mL) N (%)	GMC μg/mL
HAV	Prevaccination	118	3 (2.5)	7.7
	Mo 1	119	100 (84.0)	48.0
	Mo 7	106	106 (100)	1609.9
HAV+PCV	Prevaccination	103	1 (1)	7.6
	Mo 1	103	91 (88.3)	61.0
	Mo 7	94	93 (98.9)	1526.4
PCV→HAV	Prevaccination	122	3 (2.5)	7.8
	Mo 1	118	4 (3.4)	7.8
	Mo 8	115	113 (98.3)	1391.2

HAV group: received hepatitis A vaccine alone; HAV+PCV group: received the first dose of hepatitis A vaccine coadministered with pneumococcal conjugate vaccine; PCV→HAV group: received the pneumococcal vaccine alone followed by the first dose of hepatitis A vaccine 1 month later.

*ATP cohort for immunogenicity: those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination criteria during the study.

Safety Profile

Injection site pain was the most frequently reported AE. Pain was reported by 40.4%, 46.6%, and 53.5% of subjects in the HAV, HAV+PCV, and PCV→HAV groups, respectively. Irritability, the most frequently reported general symptom, was documented in 42.6%, 48.9%, and 57.7% of subjects in the HAV, HAV+PCV, and PCV→HAV groups, respectively.

The percentages of subjects reporting unsolicited AEs (eg, otitis media or upper respiratory tract infection) were similar (60.5%, 59.9%, and 61.1% in the HAV, HAV+PCV, and PCV→HAV groups, respectively). During the entire study period, 23 subjects (6, 7, and 10 in the HAV, HAV+PCV, and PCV→HAV groups, respectively) reported 32 serious AEs, none of which were considered to be causally related to vaccination.

DISCUSSION

One of 2 coprimary objectives was achieved. Coadministration of hepatitis A vaccine and pneumococcal conjugate vaccine did not adversely affect the immune response in terms of antihepatitis A GMC. The coprimary objective of antihepatitis A virus antibody seropositivity rates was not achieved. Although the observed difference in postvaccination antihepatitis A seropositivity rates between the groups (HAV group minus HAV+PCV group) was small (1.1%), the lower limit (−5.78%) of the 95% CI for the difference marginally exceeded the predefined noninferiority limit of −5.0%. It should be noted that a smaller than expected number of subjects completed the entire study. One hundred twenty-eight subjects per group were planned for the ATP cohort to achieve sufficient statistical power. Upon completion, there were only 106 and 94 subjects in the ATP cohorts for the HAV and HAV+PCV groups, respectively. The small difference in seropositivity between the 2 groups does not seem to be clinically significant, as 100% of subjects in the HAV group (N = 106) and 98.9% of subjects in the HAV+PCV group (N = 93) were seropositive after the second dose of hepatitis A vaccine.

The antihepatitis A seropositivity rates in the total vaccinated cohort were also analyzed. The difference in antihepatitis A virus seropositivity rates between study groups (HAV+PCV group minus the HAV group) was −0.93% and the lower limit of the two-sided standardized asymptotic 95% CI was −5.06%, as the sample size increased from 355 in the ATP cohort to 433 in the total vaccinated cohort.

The immune response to pneumococcal conjugate vaccine was not impaired by coadministration with hepatitis A vaccine. For the 7 serotypes assessed, seropositivity ranged from 98.3% to 100% at month 1 in the HAV+PCV and PCV→HAV groups. Comparable antipneumococcal antibody GMCs were observed for the 7 serotypes.

CONCLUSIONS

The antihepatitis A immune response with respect to GMC was noninferior in children who received hepatitis A vaccine coadministered with pneumococcal conjugate vaccine compared with those who received hepatitis A vaccine alone. Noninferiority of the antihepatitis A virus immune response with respect to seropositivity rates was not achieved, but the small difference between the 2 groups does not seem to be clinically meaningful, as 100% of subjects in the HAV group and 98.9% of subjects in the HAV+PCV group were seropositive after the second dose of hepatitis A vaccine. Concomitant administration may maximize vaccination opportunities at office visits and increase compliance with published recommendations.

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RESPIRATORY SYNCYTIAL VIRUS PROPHYLAXIS IN A HIGH-RISK POPULATION IN ARGENTINA

A COST-EFFECTIVENESS ANALYSIS

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Abstract: Palivizumab has proved effective in reducing hospitalization rates because of respiratory syncytial virus in vulnerable groups. In Argentina its administration is not universal because of high costs. We made a cohort study and enrolled 159 children who met the American Academy of Pediatrics recommendations but did not receive palivizumab; 26% required hospitalization for respiratory syncytial virus infection. Siblings and bronchopulmonary dysplasia were associated with higher hospitalization. For high-risk patients, one averted hospitalization was associated with costs of U.S. dollars (USD)13, 198 [number needed to treat (NNT): 4.5].

Key Words: respiratory syncytial virus, social risk, palivizumab, cost-effective analysis

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In Argentina, data on respiratory syncytial virus (RSV)-associated low respiratory infections epidemiology show hospitalization rates and disease severity higher than those reported in other studies of infants born prematurely and/or with bronchopulmonary dysplasia (BPD).[1–3] Although palivizumab (PVZ) immunoprophylaxis has proved effective in reducing hospitalization rates in most vulnerable groups,[4,5] its administration is not universal because of high costs and unequal access to health services. In our country, the cost for each vial equals 3 times the minimum income for a typical family. New drugs incorporated to the therapeutic armamentarium must be evaluated not only in terms of its efficacy and safety, but also its feasibility, efficiency, and potential coverage. Economical evaluations allow systematic and comparative analysis of public health strategies in terms of cost-benefits and far-flung consequencies.[6]

In 1999 we conducted a cost-effectiveness study based on the hypothetical administration of PVZ to a high-risk population of children assisted in the High-Risk Clinic Follow-up Program (HRCFP) of Hospital de Pediatría "Prof. Dr. JP Garrahan".[7] Data from the 42 patients included in the study showed that the cost to avoid 1 hospitalization was about U.S. dollars (USD)15,000, a figure lower than reported by others.[8,9]

As this study spanned only 2 epidemiological seasons, the cohort was kept under study to define biologic and social risk factors associated with severe RSV infection. In developing countries, in addition to the biologic risk inherent to any neonatal intensive care unit graduate, we must consider the social risk of belonging to a low-income family.[10] As the economical crisis in 2001/2002 posed special challenges to cost-benefit analysis, we decided to conduct a new cost-effectiveness trial with a greater number of patients and reflecting changing economical realities.

The main objective of our study was to explore the association between biologic and social risk factors and severe forms of RSV infection in premature infants with and without BPD, and secondarily to perform a cost-effectiveness analysis on PVZ administration for prevention of RSV-related risk-adjusted hospitalization rate.

We made a retrospective and prospective cohort study and cost-effectiveness analysis, in the HRCFP from Hospital "Prof. Dr. J.P. Garrahan" in Buenos Aires. The HRCFP database was searched; children who met the American Academy of Pediatrics recommendations[5] for PVZ administration at the beginning of May of each year (RSV season in Argentina ranges from May to September) but did not receive PVZ were considered eligible and included for analysis. Patients living more than 100 km (60 miles) from the Hospital (failure to comply with regular follow-up visits) and patients under health insurance coverage allowing PVZ administration during the study time-period were excluded. Seven consecutive years were analyzed, from 1998 to 2004. Biologic variables studied were: birth weight, gestational age, age at inclusion in follow-up program, sex, BPD, home oxygen therapy. Social variables considered were: presence of siblings or other household members under 10 years of age, maternal age, maternal education level and family with unsatisfied basic needs. The main outcome variable was hospitalization caused by RSV-related disease. Data were analyzed by Student *t* test, Mann-Whitney *U* test, or Fisher exact test, as appropriate. We performed multivariate analysis by a logistic regression model, to quantify the independent effects of biologic and social variables on the main outcome: hospitalization as a result of RSV. Statistical significance level was established at P value <0.05. Univariate and multivariate analyses were performed with STATA 8.0 for Windows.

Cost-effectiveness analysis was developed according to a public institutional perspective, based on the hypothetical administration of PVZ to the cohort of patients. Overall expenses were calculated taking into account hospitalization caused by RSV respiratory infection and in-hospital days in the pediatric ward or pediatric intensive care unit. A weighted-average estimate of the mean was used for in-hospital days. Costs information was obtained from the Department of Costs of our Hospital and are reported in 2007 USD. Per day costs in pediatric ward and pediatric intensive care unit were USD 113.94 and USD 363.21, respectively. Drug cost and drug-administration cost were calculated assuming a body weight of

TABLE 1. Cost Effectiveness Analysis in Different Risk Groups

Risk Group	Hospitalization Risk Without PVZ	Hospitalization Risk With PVZ	NNT	C/E Ratio
Without BPD With Siblings	0.28	0.06	4.5	USD 13,198
With BPD With Siblings	0.36	0.21	6.6	USD 21,152
With BPD Without Siblings	0.20	0.12	12.5	USD 43,027
Without BPD Without Siblings	0.05	0.01	25	USD 89,902

5 kg and no drug waste. Each vial of PVZ costs USD 1000 in Argentina. Health-care costs assuming PVZ prevention or no-prevention were compared. The effectiveness measure was reduction of hospitalization, according to the results from the IMpact Study.

One hundred eighty-four children met inclusion criteria, 4 were lost during follow-up and 21 were excluded because they could receive PVZ. Therefore, 159 children were enrolled in the study; of these, 41 patients (26%) required hospitalization for RSV. Hospitalization rates varied between 18% and 38% related to the year.

Median age at admission was 6 months, 77% of patients were admitted between the winter months June and July, mean length of stay was 18 days and 14 patients (34%) required mechanical ventilation for a mean of 16 days. Two patients died.

Birth weight, gestational age, intrauterine growth retardation, male gender, BPD, and need for home oxygen were not statistically different between infants who required or not RSV hospitalization. However, social risk analyses shows higher hospitalization rates for children of mothers with incomplete primary school and those with siblings under 10 years of age; besides, hospitalization risk increases as the number of siblings does: with 3 or more household under 10 years of age the odds ratio was 4.5 (95% CI: 1.8–12). In multivariate analyses we found that the odds ratio for hospitalization was more than 2-fold if there were household under 10 years of age (OR: 2.6; 95% CI: 1.3–6.5). Inadequate maternal education (OR: 2.1; 95% CI: 0.9–4.9) and presence of BPD (OR: 2.2; 95% CI: 1–5.5) showed a trend towards higher hospitalization rates. Birth weight under 1000 g did not increase risk of admission in our population (OR: 0.6; 95% CI: 0.3–1.4).

Based on these data, the cost-effectiveness analysis was performed, considering 4 risk groups: (1) patients without BPD with siblings under 10 years of age; (2) patients with BPD with siblings under 10 years of age; (3) patients with BPD without siblings under 10 years of age; and (4) patients without BPD without siblings under 10 years of age. Expected hospitalization rates and hypothetical reduction were calculated for each group. Table 1 shows data for each group: hospitalization risks with and without PVZ, number needed to treat (NNT) and cost effectiveness, reflecting amount of money needed to prevent 1 hospitalization. If results from IMpact study are used in our group of patients without BPD with siblings under 10 years of age, a 78% reduction in hospitalization rate is to be expected, meaning USD 13,198 to avoid 1 hospitalization (NNT = 4.5). For the group with BPD with siblings under 10 years of age, a 39% reduction in hospitalization rate is to be expected, and figure would be USD 21,152 (NNT = 6.6).

The purpose of a cost-effectiveness analysis in health care is to help decision-makers determine how to allocate resources among a number of competing needs to maximize health outcomes from a limited budget. In Argentina, health economic evaluations have been recently developed through governmental and nongovernmental organizations that perform these analyses upon request for introduction of new high-cost technologies. Availability for these evaluations is related with special programs and at present times definition thresholds for cost effective and socially accepted technologies for Argentina are still lacking. In our country, PVZ introduction has generated controversies. Data from this study allow us to confirm that there is a high hospitalization rate (26%) because of RSV-related disease in the high-risk population described and that hospitalized infants show severe clinical condition and a large proportion requires mechanical ventilation and prolonged hospitalization. Besides, social factors, namely number of siblings, are strongly associated with serious infection by RSV, and therefore, biologic risk factors currently used in developed countries are not enough to define the risks for RSV hospitalization in low socioeconomic high risk groups in developing nations like Argentina. We speculate that prospective studies including socioeconomic variables may provide more information to develop better grounded recommendations for prophylaxis with high-cost drugs in underdeveloped countries.

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HUMAN HERPESVIRUS 8 SEROPREVALENCE AMONG CHILDREN AND ADOLESCENTS IN THE UNITED STATES

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Abstract: We measured human herpesvirus-8 antibodies (K8.1 and orf73 enzyme immunoassays) in 4166 children, aged 6–17 years, in a U.S. cross-sectional survey. Forty-six were K8.1 seropositive (weighted seroprevalence: 1.1%) and 20 were orf73 seropositive (weighted seroprevalence 0.4%). K8.1 seropositivity was associated with asthma (odds ratio: 6.3; 95% confidence interval: 2.4–16.9) and hay fever (3.5; 1.1–11.0), and there were borderline associations with measures of crowding and low socioeconomic status.

Key Words: human herpesvirus 8, HHV-8, Kaposi sarcoma-associated herpes virus, KSHV, children, adolescents, U.S., asthma, hay fever, socioeconomic status, familial clustering

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Human herpesvirus 8 (HHV-8) is associated with the development of Kaposi sarcoma, primary effusion lymphoma, and multicentric Castleman disease. HHV-8 seroprevalence varies by geographic location but is low in most developed countries. Among adults in the general U.S. population, HHV-8 seroprevalence has been estimated to range from 2% to 7%.[1,2] In the United States, HHV-8 infection is thought to mainly occur in adulthood and is associated with high-risk sexual behaviors.[2]

HHV-8 infection among children has been less well studied. Exposure to saliva seems to be the main mode of HHV-8 acquisition, and familial clustering has been reported in Africa.[3] In Africa, HHV-8 seroprevalence in children is high and has been associated with low socioeconomic status.[4] A study in South Texas reported high HHV-8 seroprevalence (26%) among 123 healthy children aged 4–13 years.[5] Other studies investigating HHV-8 seropositivity were limited to U.S. children and adolescents infected with, or at high risk of infection with, human immunodeficiency virus[6,7] We used sera and data from the third National Health and Nutrition Examination Survey (NHANES III) to study HHV-8 epidemiology in a large representative sample of U.S. children and adolescents.

METHODS

NHANES III, conducted by the National Center for Health Statistics during 1988–1994, is a cross-sectional survey of the U.S. noninstitutionalized civilian population. Household interviews, standardized physical examinations, and biologic samples were collected.[8] NHANES III included 39,365 individuals randomly sampled through a complex, multistage probability design. Non-Hispanic blacks and Mexican Americans were oversampled. Of the 7368 children and adolescents aged 6–17 years enrolled in NHANES III, 6942 were examined and 5771 had phlebotomy performed. The present report is based on the 4166 subjects aged 6–17 years for whom surplus serum was available for testing.

HHV-8 antibody testing was performed during a period of 6 months using enzyme immunoassays (EIAs) to detect IgG antibodies to the HHV-8 K8.1 structural glycoprotein, expressed during lytic replication, and the orf73 protein (also known as LANA), expressed during latent infection.[2] To account for plate-to-plate variability, we adjusted optical density (OD) measurements for samples by regressing them against the mean OD of the positive controls on the respective EIA plate.[2] Cutoffs were based on the distribution of the residuals from the regression models, and were chosen to be high to increase the specificity and positive predictive value of the EIA results (termed "conservative cutoffs" throughout). As a consequence, we may have underestimated the prevalence of HHV-8 infection in children and adolescents in the United States. In a sensitivity analysis, we used previously published cutoffs (termed "alternative cutoffs").[2] These alternative cutoffs yielded higher seroprevalence estimates but did not fully account for plate-to-plate variability in the EIA results.

For each EIA, HHV-8 seroprevalence was calculated using the conservative and the alternative cutoff to provide a range. To compare seroprevalence in subjects differing by demographic variables and medical history, we present odds ratios (ORs) and 95% confidence intervals (CIs) based on logistic regression. Individuals included in this report were older than the NHANES III children and adolescents who lacked surplus serum ($P < 0.001$), and the 2 groups differed by race/ethnicity ($P < 0.001$). Because these differences could have introduced bias, we generated a new set of sample weights by poststratifying the standard NHANES III sample weights, which reflect NHANES III sampling probabilities, so that the new weighted totals matched corresponding totals for the U.S. population by age, gender, and race/ethnicity. All analyses used these new sample weights. SAS-callable SUDAAN, version 9.0.1 (Research Triangle Park, NC, 2005) was used for all analyses.

Children who were tested for HHV-8 antibodies were linked to other sampled household members in NHANES III who had an HHV-8 determination. We used this information to examine whether K8.1 seropositive children had other household members (children or adults[2]) who were also seropositive. No child who was orf73 seropositive had a family member who was also orf73 seropositive.

RESULTS

Using the conservative assay cutoff, 47 of the 4166 children and adolescents were K8.1 seropositive, yielding a weighted seroprevalence of 1.1% (95% CI: 0.7–1.4%). Only 20 children and adolescents were orf73 seropositive according to the conservative cutoff (weighted seroprevalence: 0.4%; 95% CI: 0.2–0.6%). Five children tested seropositive for both K8.1 and orf73 ($\kappa = 0.14$). Using the alternative cutoffs, K8.1 seroprevalence and orf73 seroprevalence increased to 5.7% (95% CI: 4.9–6.3%) and 6.1% (95% CI: 5.3–6.3%), respectively.

With the conservative cutoff, K8.1 seroprevalence did not vary by gender, age, or race/ethnicity (Table 1). K8.1 seroprevalence was approximately 2-fold higher, albeit not statistically significant, among children and adolescents in households with fewer than 6 rooms, more than 5 people, an unemployed reference person, or less than $10,000 annual income (Table 1).

Children and adolescents with asthma or hay fever were significantly more likely to be K8.1 seropositive than individuals without these conditions (ORs: 6.3 and 3.5, respectively; Table 1). Adjusting for age, sex, race/ethnicity, household smoking, or socio-

TABLE 1. Subject Characteristics and Associations With HHV-8 Seropositivity Among 4166 U.S. Children Using the Conservative Assay Cutoffs

Characteristic	Total	K8.1 Enzyme Immunoassay		Orf73 Enzyme Immunoassay		Either Enzyme Immunoassay	
		No. Seropositive (Weighted %)	Weighted OR (95% CI)	No. Seropositive (Weighted %)	Weighted OR (95% CI)	No. Seropositive (Weighted %)	Weighted OR (95% CI)
Gender							
Male	2053	20 (0.88)	1.0	8 (0.34)	1.0	27 (1.20)	1.0
Female	2113	27 (1.33)	1.5 (0.6–4.0)	12 (0.44)	1.3 (0.5–3.6)	35 (1.63)	1.4 (0.6–3.0)
Race/ethnicity							
Non-hispanic white	1024	10 (1.4)	1.0	5	1.0	15 (1.41)	1.0
Non-hispanic black	1465	19 (1.8)	1.3 (0.5–3.2)	11	3.1 (0.8–12.0)	25 (1.82)	1.3 (0.6–2.8)
Mexican American	1458	17 (1.4)	0.8 (0.3–1.8)	4	1.8 (0.4–9.2)	21 (1.44)	1.0 (0.5–2.0)
Other	219	1 (0.9)	0.8 (0.1–6.8)	0	—	1 (0.91)	0.6 (0.1–5.2)
Age, yrs							
6–7	747	7 (1.18)	1.0	2 (0.37)	1.0	9 (1.55)	1.0
8–9	780	8 (0.31)	0.3 (0.1–1.1)	3 (0.29)	0.8 (0.1–6.2)	11 (0.61)	0.4 (0.1–1.4)
10–12	1038	16 (1.73)	1.5 (0.3–7.1)	8 (0.80)	2.2 (0.5–9.4)	21 (2.22)	1.5 (0.4–5.1)
13–17	1601	16 (1.03)	0.9 (0.2–3.2)	7 (0.22)	0.6 (0.1–3.7)	21 (1.22)	0.8 (0.3–2.3)
Family reference person employed							
Yes	2937	28 (0.87)	1.0	11 (0.29)	1.0	38 (2.41)	1.0
No	1043	18 (1.97)	2.3 (0.7–7.8)	7 (0.56)	1.9 (0.5–7.8)	22 (1.15)	2.4 (1.1–5.1)
Family yearly income							
$0–$9999	803	15 (1.70)	1.0	4 (0.28)	1.0	16 (1.76)	1.0
$10,000–$19,999	1076	11 (0.57)	0.3 (0.1–1.3)	5 (0.51)	1.8 (0.4–8.7)	15 (1.05)	0.6 (0.1–2.6)
$20,000–$44,999	1435	14 (1.38)	0.8 (0.3–2.4)	3 (0.27)	1.0 (0.2–4.9)	17 (1.65)	0.9 (0.3–2.6)
≥$50,000	488	3 (0.76)	0.4 (0.1–2.1)	5 (0.31)	1.1 (0.3–4.9)	8 (1.07)	0.6 (0.1–2.3)
Family size							
<5 people	1840	14 (0.74)	1.0	11 (0.44)	1.0	24 (1.16)	1.0
≥5 people	2326	33 (1.52)	2.1 (0.8–5.8)	9 (0.33)	0.8 (0.2–2.8)	38 (1.69)	1.5 (0.6–3.4)
Household size							
<5 people	1814	14 (0.74)	1.0	11 (0.44)	1.0	24 (1.18)	1.0
≥5 people	2352	33 (1.50)	2.0 (0.7–5.7)	9 (0.33)	0.7 (0.2–2.7)	38 (1.67)	1.4 (0.6–3.4)
Rooms in house							
<6 rooms	2063	30 (1.56)	1.0	7 (0.36)	1.0	34 (1.73)	1.0
≥6 rooms	2103	17 (0.85)	0.5 (0.2–1.6)	13 (0.40)	1.1 (0.4–3.6)	28 (1.23)	0.7 (0.3–1.8)
Household density							
<1 person per room	2313	19 (0.74)	1.0	14 (0.44)	1.0	31 (1.18)	1.0
≥1 person per room	1853	28 (1.50)	1.4 (0.5–4.3)	6 (0.33)	0.8 (0.2–3.6)	31 (1.67)	1.1 (0.4–2.9)
Urbanization							
Urban	1966	24 (1.12)	1.0	8 (0.19)	1.0	30 (1.29)	1.0
Rural	2200	23 (1.08)	1.0 (0.3–2.9)	12 (0.55)	2.9 (0.9–8.9)	32 (1.51)	1.2 (0.5–3.0)
Asthma							
No	3782	39 (0.71)	1.0	19 (0.43)	1.0	54 (4.34)	1.0
Yes	383	8 (4.34)	6.3 (2.4–16.9)	1 (0.07)	0.2 (0.0–1.4)	8 (1.06)	4.3 (1.6–11.2)
Hay fever							
No	3922	40 (0.90)	1.0	17 (0.36)	1.0	53 (1.18)	1.0
Yes	244	7 (3.08)	3.5 (1.1–11.0)	3 (0.66)	1.8 (0.4–8.7)	9 (3.68)	3.4 (1.3–8.9)
Cold/flu in past 12 mo							
No	908	7 (0.56)	1.0	3 (0.35)	1.0	9 (0.86)	1.0
Yes	3258	40 (1.23)	2.2 (0.5–9.8)	17 (0.40)	1.1 (0.2–6.5)	53 (1.54)	1.7 (0.8–3.9)
Sinusitis in past 12 mo							
No	3465	39 (0.93)	1.0	16 (0.34)	1.0	52 (2.04)	1.0
Yes	695	8 (1.74)	1.9 (0.7–5.5)	4 (0.57)	1.7 (0.4–6.7)	10 (1.24)	1.7 (0.4–6.7)

Totals do not always sum to n = 47 K8.1 seropositive or n = 20 orf73 seropositive subjects, or to n = 4166 for the total population, because of missing questionnaire data.

economic status variables did not alter these associations (data not shown). K8.1 seropositivity was higher among children and adolescents who had a cold/flu or sinusitis in the 12 months before the survey, although these relationships were not significant (Table 1). Among K8.1 seropositive subjects, K8.1 antibody concentration was significantly higher in the subset who had had sinusitis (mean OD: 2.50 versus 2.10; $P = 0.005$) and nonsignificantly higher in those who had had cold/flu (mean OD: 2.20 versus 1.97; $P = 0.29$) in the preceding 12 months.

With the conservative cutoff, there were very few orf73 seropositive subjects and no associations were significant (Table 1). With the alternative cutoffs, K8.1 seroprevalence seemed to increase with increasing age (P for trend = 0.04, data not presented). Only the association between asthma and K8.1 seropositivity remained statistically significant (OR: 2.4; 95% CI: 1.2–4.9). When we considered individuals as seropositive if they were seropositive according to either assay, the results generally mirrored those using the K8.1 enzyme immunoassay alone (Table 1).

Of the 42 K8.1 seropositive children and adolescents who had a family member tested for K8.1 antibody, 4 (9.5%) had at least 1 family member who was K8.1 seropositive. In comparison, of the 3677 K8.1 seronegative children and adolescents who had a family member tested, 166 (4.5%) had a family member who was K8.1 seropositive.

DISCUSSION

This is the largest study to investigate HHV-8 epidemiology among children and adolescents in the general U.S. population. K8.1 seroprevalence was significantly higher in children with asthma, hay

fever, and, possibly, sinusitis. Although not statistically significant, our analysis suggested that K8.1 seropositivity was also associated with lower socioeconomic circumstances.

The major limitation of this study, as for other studies of HHV-8 epidemiology, is the lack of standard serological assays for HHV-8 infection. Although we used established EIAs to detect IgG antibodies to 2 viral proteins, variability in the testing results over time required us to take a new approach in standardizing the results across assay plates.[2] We expect that this approach reduced the assay variability and increased the reliability of comparisons between groups of subjects, but the sensitivity and specificity of our assays under this approach are unknown. The poor agreement between the 2 EIAs observed in this study ($\kappa = 0.14$) probably reflects the low prevalence of HHV-8 in the general U.S. population as well as the assay performance. In addition, we chose highly conservative cutoffs to increase the specificity of the EIAs, which may have caused us to underestimate HHV-8 seroprevalence. HHV-8 seroprevalence was higher using the alternative cutoffs, but these results would be expected to be more variable, less specific, and associated with a higher false positive rate than the conservative cutoffs.

Because the EIAs have uncertain sensitivity and specificity, we are reluctant to interpret our seroprevalence results as estimates of the true prevalence of HHV-8 infection among U.S. children and adolescents. As expected, K8.1 and orf73 seroprevalence were lower than we previously reported for U.S. adults using these same assays,[2] possibly explained, in part, by an increase in prevalence of HHV-8 with age. Our estimates were also much lower than that reported in predominantly Hispanic children aged 4–13 years in a study from South Texas,[5] which could reflect differences in the study population or HHV-8 testing. We did not see any difference in HHV-8 seroprevalence between white and Mexican-American children or adolescents in our study. A limitation is that our findings represent HHV-8 seroprevalence between 1988 and 1994, and changes in HHV-8 epidemiology may have occurred during the last 14 years.

Evidence for associations with socioeconomic status and familial clustering have been demonstrated in studies from areas with high HHV-8 seroprevalence.[3] Although the mode of HHV-8 transmission is unknown, virus can be detected in saliva, and it is plausible that poverty or crowded living conditions could facilitate HHV-8 transmission by this route. Although we found no significant associations, HHV-8 seroprevalence seemed to be higher in children with lower socioeconomic status. Our study also suggested that familial clustering can occur. However, our findings regarding familial clustering should be considered descriptive because the low seroprevalence of HHV-8 and the complexity of the survey design prevented us from conducting a formal statistical analysis.

This is the first study to report an association of HHV-8 seropositivity with asthma and hay fever. We also observed nonsignificantly higher seroprevalence in subjects who had had cold/flu or sinusitis in the previous 12 months. In a prospective study, 5 of 6 Egyptian children with primary HHV-8 infection presented with fever and skin rash, followed by a secondary upper or lower respiratory infection.[9] We speculate that the association between K8.1 seropositivity and respiratory illnesses may be due, at least in part, to symptoms related to recent primary infection. However, these associations could possibly reflect increased vulnerability to HHV-8 infection among children with respiratory disease, or mere chance, because we tested multiple associations.

In conclusion, our study found suggestive evidence of household transmission of HHV-8 among U.S. children and adolescents, although the results were not statistically significant. The relationships of HHV-8 seropositivity with respiratory conditions and hay fever warrant additional evaluation.

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ANAPLASMA PHAGOCYTOPHILUM INFECTION IN A CHILD

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Abstract: This is the first case of *Anaplasma phagocytophilum* infection described in Cyprus. A 9-year-old girl was infected after a tick-bite. The infection was diagnosed by molecular-based detection of the bacterium in 2 blood samples. The polymerase chain reaction product was sequenced, revealing a novel strain of *Anaplasma phagocytophilum*.

Key Words: *Anaplasma phagocytophilum,* child, PCR

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Anaplasmoses are tick-borne emerging zoonoses (caused by bacteria within the family Anaplasmataceae). The causative agents are maintained through enzootic cycles between ticks and animals.[1]

CASE REPORT

A 9-year-old girl was admitted to the Department of Pediatrics of Archbishop Makarios Hospital, Nicosia, Cyprus, on February 7, 2006 with a 3.5-month history of recurrent fever up to 38°C. The patient had been hospitalized at a provincial hospital for the first time in October when she received oral doxycycline and ceftriaxone for a total of 10 days and was discharged afebrile. Her parents reported that they had removed a tick from her neck while she was playing with their dog a few weeks before the beginning of the symptoms in October 2005. The child complained of no other accompanying symptoms and was otherwise feeling well. No abnormal findings were revealed on physical examination.

The patient's peripheral blood revealed an intense granulation of neutrophils and mild shift to the left. The liver enzyme concentrations were mildly raised, as were C-reactive protein (13.7 mg/dL, neg. <0.5) and erythrocyte sedimentation rate (58 mm). Testing of the patient's serum for antinuclear antibodies, anti-DNA antibodies, and rheumatoid factor were negative. Serologic tests for cytomegalovirus, herpes-simplex virus 1, Epstein-Bar virus, varizella-zoster virus, parvovirus, and adenovirus were also negative. Molecular tests [polymerase chain reaction (PCR)] for the detection of the above viruses in blood were negative. The blood culture and the urine microscopy and culture were negative. A bone marrow biopsy examination did not show malignancy. A magnetic resonance imaging scan of the thorax and abdomen was normal as was the echocardiogram. The fever resolved 9 days after the start of doxycycline and the child was discharged on March 16, 2006.

METHODS

During her last hospitalization, 2 whole blood samples and 2 serum samples were sent to the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, Crete, Greece (World Health Organization Collaborating Center). The early and late blood samples were drawn at 15-day intervals, during the patient's fever spikes.

The serum samples were examined by immunofluorescence antibody using commercially available kits (Focus Diagnostics), for the presence of antibodies against *Brucella*, *Anaplasma phagocytophilum*, *Rickettsia conorii*, *Rickettsia typhi*, *Coxiella burnetii*, *Borrelia burgdorferi*, *Bartonella henselae,* and *Bartonella* qu*intana*.

DNA was extracted from the 2 whole blood samples using the QIAmp blood minikit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. The extracts were used as targets for PCR amplification of *Bartonella* species,[2] *Anaplasma* species,[3] *C. burnetii*,[4] and *Rickettsia* species.[5] For the detection of *B. burgdorferi* a commercially available kit was used (Maxim Biotch, Inc.).

RESULTS

On immunofluorescence, no titers of diagnostic significance were found for the above pathogens. The first 2 whole blood samples gave a positive result for *Anaplasma* species by PCR amplification. All whole blood samples gave negative results for the remaining pathogens.

The PCR amplification products were purified and the nucleic acid sequences of both strands were determined by sending the products to Qiagen. The sequence alignment was given a GenBank accession number (DQ822072) and was compared against the sequences DQ458808, DQ449948, AY055469, AY527214, AY144728, DQ105667, AF481855, which all correspond to *A. phagocytophilum* strains, revealing a 98% sequence homology. The homology search was performed using the BLAST program of the National Center for Biotechnology Information, and an evolutionary tree was constructed using the Clusta-w software (Fig. 1, available online only).

Epidemiology. An epidemiologic survey was carried out in the village where the patient lived. We collected 22 samples from the child's parents and from neighbors, as well as 15 samples from animals in the nearby region (3 samples from dogs, 5 samples from goats, and 7 samples from sheep). Thirty-four ticks parasitizing the animals were removed, classified, homogenized, and tested by PCR in the same manner as blood samples. Dogs were parasitized by 12 ticks of the genus *Rhipicephalus sanguineus* and by 1 *Rhipicephalus turanicus*, whereas 21 ticks of the genus *Hyaloma anatolicum excavatum* were collected from sheep. There were no ticks found on goats.

All blood samples were tested for IgG antibodies against *A. phagocytophilum*, as described above. The whole blood samples of the animals were pooled and tested by PCR for the presence of the pathogen, following the technique described above.

Twelve (55%) of the human samples tested positive for antibodies, the highest titer being 1/6400. The patient's father had anti-*Anaplasma* antibodies at a titer of 1/100, whereas the mother had a titer of 1/3200. The mother reported having contact with the same dog as her daughter. Neither parent had clinical symptoms either before or at the day of blood sampling, as was true for the rest of the humans who tested positive.

Of the animal samples, 3 of the 5 goats (highest titer 1/100) and 6 of the 7 sheep (highest titer 1/400) tested positive for antibodies against the pathogen.

The pool corresponding to the goats tested positive by PCR, whereas sheep and dogs were negative. None of the animals presented any clinical signs of illness. The PCR amplification product was sequenced as above and revealed a 97% similarity with various strains of *Anaplasma ovis*, *Anaplasma centrale*, *Anaplasma marginale,* and *A. phagocytophilum*, indicating the presence of *Anaplasma* species in the region where the little girl was living.

None of the ticks tested positive by PCR for *A. phagocytophilum*.

DISCUSSION

The first case of clinically recognized human granulocytic anaplasmosis was described in the United States in 1994.[6] The disease emerged in Europe in 1997.[7] Since then, 22 cases have been laboratory confirmed in Holland, Sweden, Slovenia, Spain, Austria, Poland, and France. The case we report concerns a 9-year-old child and adds to knowledge about the disease since the first report of anaplasmosis in children.[8] The diagnosis in our child was based on a positive PCR, in accordance with the case definition for human anaplasmosis.[9] The clinical and laboratory findings of our patient were consistent with those described in human anaplasmosis.[10,11] She had persistently high lactate dehydrogenase values and the fever did not resolve for at least 5 months after the onset of symptoms. No coinfection was diagnosed, despite the fact that infections with tick-borne encephalitis and/or *B. burgdorferi* have been described.[12,13] The absence of antibodies to *A. phagocytophilum* may, in part, be explained by the prolonged administration of the antibiotic, producing a masking effect as described by Young and Klein,[14] who reported a complete absence of *A. phagocytophilum* antibodies in the presence of a positive PCR up to 6 months after hospitalization (that patient became afebrile after administration of doxycycline). A positive PCR targeting the 16s rRNA gene, together with the absence of any antibodies to *A. phagocytophilum* has also been reported by others.[15] We could not identify any morulae when staining the peripheral blood, even when blood was drawn during fever spikes, enhancing the current speculation that this method is not sensitive. Our sequenced PCR product revealed a similarity (98%) to other *A. phagocytophilum* strains. The 97% similarity with *Anaplasma* species of the PCR product in goats indicates the presence of *Anaplasma* in the above surveyed region.

The clinicians should always have at the back of their minds the possibility of an *A. phagocytophilum* infection in cases of children with fever of unknown origin and a history of tick-bite.

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VENTRICULITIS AND CHOROID PLEXITIS CAUSED BY MULTIDRUG-RESISTANT *NOCARDIA PSEUDOBRASILIENSIS*

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Abstract: We describe a case of ventriculitis and choroid plexitis caused by a multidrug-resistant *Nocardia pseudobrasiliensis* in an immunocompetent child. Difficulties establishing an etiologic diagnosis, inconsistencies of antibiotic susceptibility testing, and the side effects of various antimicrobials presented challenges to her treatment and eventual favorable outcome.

Key Words: *Nocardia pseudobrasiliensis*, nocardiosis, ventriculitis, choroid plexitis, central nervous system

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Nocardia include a group of soil saprophytes noted for causing opportunistic infections, often among immunocompromised individuals. *Nocardia* are delicately filamentous, weakly Gram-positive, strictly aerobic organisms related to corynebacteria and mycobacteria within the order Actinomycetales. *N. pseudobrasiliensis* has only recently been distinguished from *N. brasiliensis*, and isolates of this new species have been associated with invasive disease, including brain, kidney, bone, and disseminated infections.[1,2] A few case reports have noted clinical experience in adults,[3,4] but no cases have been described in children.

CASE REPORT

A 9-year-old white female presented to the Children's Hospital of Illinois with a 4-day history of fever, headache, vomiting, and photophobia. The physical examination revealed nuchal rigidity. Her medical history was significant for tethered spinal cord; therefore, the lumbar puncture was not initially performed. She had no environmental exposure to soil, compost, or decaying vegetation. There was no travel history outside Illinois. The patient was treated with intravenous ceftriaxone and vancomycin for presumed bacterial meningitis. Cranial computed tomography (CT) was normal, and magnetic resonance imaging of the spine revealed a tethered cord at L4-5. A lumbar puncture was then performed under fluoroscopy. The cerebrospinal fluid (CSF) contained 1790 white blood cells/mm^3 with 68% neutrophils, 21% lymphocytes, 11% monocytes; 11 red blood cells/mm^3; 84 mg/dL protein; and 34 mg/dL glucose. Gram-stained smears of CSF and bacterial cultures were negative. Vancomycin was subsequently discontinued, and she was continued on ceftriaxone for a total of 10 days.

Five days after discharge, the patient presented again with a 2-day history of fever and severe headache. Cranial CT and magnetic resonance imaging scans now revealed inflammatory ventriculitis and choroid plexitis with entrapment of the right temporal horn (Figs. 1, 2; available online only). She underwent ventriculostomy placement to alleviate the obstruction. Empiric antimicrobial therapy with intravenous vancomycin and meropenem was initiated. Analysis of the CSF from the ventricles obtained during the ventriculostomy placement revealed 867 white blood cells/mm^3 with 65% neutrophils, 23% lymphocytes, 12% monocytes; 4800 red blood cells/mm^3; 139 mg/dL protein; and 46 mg/dL glucose. No microorganisms were seen on the Gram, fungal, and acid-fast stained smears. The CSF culture was negative for bacteria, fungi, and mycobacteria.

Ventricular fluid taken from the ventriculostomy on the third day after admission grew Gram-positive filamentous branching bacilli. A modified Kinyoun acid-fast stained smear was negative.

The organism was identified as a presumptive *Actinomyces* species by the Gram stain appearance, colonial morphology, and the presence of sulfur granules from the culture broth. Vancomycin and meropenem were discontinued, and intravenous ampicillin was started. One of the ventricular fluid culture isolates was sent for species-level identification and susceptibility pattern to Laboratory A.

Further evaluation revealed a normal CT scan of chest and abdomen. Tests of immunologic function including HIV, quantitative measurement of serum immunoglobulins, lymphocyte subsets, and neutrophil oxidative burst were normal. The patient was discharged home without neurologic sequelae after 3 weeks of hospitalization to continue intravenous antibiotic therapy.

About 4 weeks after discharge, a cranial CT scan was performed and not only revealed findings consistent with ventriculitis, choroid plexitis, entrapment of occipital horn, but also showed an interval progression of cerebral edema. Physical examination revealed bilateral papilledema and ptosis of right eye. Meanwhile, Laboratory A reported that the isolate was identified as *N. pseudobrasiliensis* based on 16S rRNA gene sequence analysis and biochemical profile. The antimicrobial regimen was changed to intravenous trimethoprim-sulfamethoxazole (TMP-SMX), meropenem, and amikacin, pending the results of drug susceptibility testing. Microdilution minimal inhibitory concentration (MIC) testing performed at Laboratory A revealed susceptibility to amikacin, ciprofloxacin, and clarithromycin, and resistance to TMP-SMX, ceftriaxone, minocycline, and imipenem. Based on this information, TMP-SMX and meropenem were discontinued, and ciprofloxacin and linezolid were started intravenously.

Given the finding of multidrug resistance, the antimicrobial susceptibility testing were repeated at Laboratory B and at the Nocardia Research Laboratory, Department of Microbiology, University of Texas Health Center at Tyler, TX. The susceptibility patterns of these isolates (Table 1) performed in those laboratories were similar to each other except for discrepancies between the results of susceptibility to sulfamethoxazole. The isolate was reported to be susceptible with an MIC of 32 μg/mL at Laboratory B and resistant with an MIC of 64 μg/mL at University of Texas Health Center.

Linezolid and amikacin were continued. Ciprofloxacin was subsequently changed to gatifloxacin, because it was reported as resistant to ciprofloxacin but susceptible to gatifloxacin. TMP-SMX was added to the regimen based on the result of susceptibility to sulfamethoxazole performed at Laboratory B. Her clinical status improved. However, after 3 weeks of the therapy with this regimen, she developed pancreatitis (lipase 1070 U/L) attributed to a proton-pump inhibitor (esomeprazole), which was discontinued. Despite the decline of lipase concentrations, she continued having nausea and vomiting. Further evaluation revealed metabolic acidosis with a high anion-gap (18–24 mmol/L) and elevated serum lactate values (4–15 mmol/L). Physical examination showed peripheral neuropathy. Therefore, linezolid was discontinued. Shortly thereafter, the amikacin was stopped because of renal insufficiency (creatinine 1.6 mg/dL), and the gatifloxacin was discontinued because the ECG showed a prolonged QTc interval. TMP-SMX was continued as the only antimicrobial agent.

The patient improved clinically with a progressive recovery of neurologic deficits during her 2 months stay in the hospital. She was discharged home to receive intravenous TMP-SMX (18 mg/kg/d trimethoprim), which was changed to oral TMP-SMX after 12 weeks. She has continued to do well clinically. A cranial CT done 13 months after the beginning of effective therapy demonstrated slight interval progression of a temporal lobe cyst, which was thought to be secondary to white matter gliosis. She underwent a stereotactic decompression of the cyst, and cultures of the cystic fluid were sterile.

TABLE 1. Summary of Antimicrobial Susceptibility Testing Results

Antibiotic	Antibiotic MIC* (Susceptibility Determination)		
	Microdilution Plates (Trek Diagnostics)		Broth Microdilution UTHCT†
	Laboratory A	Laboratory B	
Amikacin	8 (S)	8 (S)	8 (S)
Ciprofloxacin	0.025 (S)	0.5‡ (S)	1 (S)
Gatifloxacin	—	≤0.12 (S)	—
Linezolid	—	0.5 (S)	2 (S)
TMP-SMX	8/152 (R)	—	—
Sulfamethoxazole	—	32 (S)	64 (R)
Ceftriaxone	>64 (R)	>124 (R)	>32 (R)
Imipenem	>64 (R)	>32 (R)	>32 (R)

*In micrograms per milliliter.
†Mycobacteria/Nocardia Laboratory, University of Texas Health Center at Tyler, TX.
‡On initial testing, the isolate was reported as ciprofloxacin resistant, but susceptible on a repeat testing.

DISCUSSION

Infection caused by *N. pseudobrasiliensis*, a newly described species that was once thought to be *N. brasiliensis*, has been associated with invasive infections such as disseminated, central nervous system (CNS) or pulmonary nocardiosis.[1,2] As described in this report, the patient had severe CNS involvement, ventriculitis, and choroid plexitis. CNS nocardiosis involving the choroid plexus has been reported,[5,6] and nocardiosis should be considered in the differential diagnosis when approaching a patient with enhancing lesion of the choroid plexus.

The case presented here illustrates a pitfall in a diagnosis of *Nocardia* infection leading to inappropriate antimicrobial therapy. Two specimens from the patient's ventricular fluid were initially identified as *Actinomyces* species, based on the Gram stain appearance, colonial morphology, the presence of sulfur granules, and negative modified acid-fast stain. The distinction between *Actinomyces* and *Nocardia* species is of extreme clinical importance because of the different antimicrobials required for treatment. On the Gram stain, *Nocardia* and *Actinomyces* species are morphologically indistinguishable. *Nocardia* species can exhibit a distinct variably acid-fast property when decolorized with 1% sulfuric acid. This modified acid-fast stain can be useful to guide a diagnosis, if positive. Identification of sulfur granules is said to be a hallmark of actinomycosis, but others report that sulfur granules can also be seen with nocardiosis.[7] Futhermore, growth under aerobic conditions should arouse the suspicion of *Nocardia* organisms. In contrast, *Actinomyces* are bacteria with anaerobic or microaerophilic growth requirements. Although *Actinomyces* are also facultative anaerobic organisms, they often fail to grow aerobically in primary culture.

Nocardia species can vary in their antimicrobial susceptibility patterns. Thus, therapeutic efficacy in individual patients depends on the species and on in vitro susceptibility studies. The majority of *N. pseudobrasiliensis* originally described by Wallace et al[1] were susceptible to ciprofloxacin (95%), clarithromycin (91%), cefotaxime (78%), and ceftriaxone (69%). However, 34 of 37 isolates (92%) were resistant to a 25 μg TMP-SMX disk by disk diffusion, but most of these isolates (29 of 31 isolates, 94%) were susceptible to sulfamethoxazole by MIC determination (MIC ≤32 μg/mL). This finding may reflect the low concentration of sulfa in the disk resulting in the discrepancy between the disk diffusion and MIC results.[1]

No established optimal regimens exist for the treatment of multidrug resistant nocardiosis. Linezolid is an attractive therapeutic

agent, because it has excellent oral bioavailability and high activity against most of the *Nocardia* isolates.[8] Linezolid is relatively safe, but its use has been associated with anemia and thrombocytopenia,[9] and less commonly with lactic acidosis resulting from a disruption of mitochondrial function.[10] As illustrated in this case, the patient developed serious adverse side effects to the various antimicrobial agents, including lactic acidosis (linezolid), a prolonged QTc interval (gatifloxacin), and renal insufficiency (amikacin) which precluded the use of a multidrug regimen.

We used TMP-SMX as monotherapy, although no studies have systematically correlated in vitro susceptibility with clinical outcomes. Despite resistance to SMX, the combination TMP-SMX demonstrated synergy in vitro as shown by 4-fold or greater decrease in the MIC when 2 agents were combined.[11] More interestingly, TMP-SMX in combination with other antimicrobial agents can be effective, despite apparently reduced in vitro susceptibility to TMP-SMX.[12–14] Several factors influencing susceptibility of *Nocardia* isolates to TMP-SMX including the duration of incubation and the FIC index (fractional inhibitory concentration of each component of the mixture) were reported, such that the TMP-SMX ratio in the commercial fixed-dose combination disk contained too little trimethoprim to be optimal for *Nocardia*.[15,16] We decided to maintain TMP-SMX because of the clinical improvement of the patient and the difficulty in interpretation of susceptibility results.

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FATAL NEONATAL MYOCARDITIS CAUSED BY A RECOMBINANT HUMAN ENTEROVIRUS-B VARIANT

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Abstract: We report a case of fatal myocarditis in a newborn infant who was infected with a human enterovirus detected by throat culture and RT-PCR for viral RNA in plasma. Whole genome sequence analysis revealed the virus to be a genomic chimera that likely arose from recombination between coxsackievirus B3 and two recently identified enteroviruses, EV 86 and EV97.

Key Words: enterovirus, neonate, recombination, myocarditis

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More than 80 serologically distinct viruses make up the *enterovirus* genus within the family *Picornaviridae.* These enteroviruses are currently classified into 5 species: *Human enteroviruses* (HEV) A–D and the polioviruses.[1] The 6 group B coxsackieviruses (CVB) are members of the HEV-B species and are leading causes of myocarditis and central nervous system infections in the United States.[2] CVB are also associated with substantial morbidity in newborns, and neonatal case-fatality rates of 18–40% have been estimated for CVB2, CVB3, and CVB4.[3] Recent reports have indicated that recombination within the HEV-B species is common, leading to enhanced genetic diversity.[4–6] However, the impact of recombination on the virulence of these agents is unclear, and only a few full length sequences are available.

Here we report a case of fatal infection with a recombinant enterovirus with genome comprising sequences apparently derived from coxsackievirus B3 (CVB3) and more recently identified enteroviruses, including enteroviruses 86 and 97.

THE CASE

The patient was a full term male infant born in October 2005 by vaginal delivery after an uncomplicated pregnancy with a birth weight of 3.5 kg. The parents had been well in the month preceding his birth, but a sibling of the infant had had an episode of diarrhea approximately 3 weeks earlier. A newborn screening study on the

first day of life revealed no evidence of congenital adrenal hyperplasia, galactosemia, hemoglobinopathies, or errors of lipid or amino acid metabolism. On the third day of life, he developed fever (38.8°C) and irritability and was admitted to the Mattel Children's Hospital at UCLA (MCH). A lumbar puncture revealed normal cerebrospinal fluid (2 WBC and 9 RBC/μL, protein 59 mg/dL, and glucose 63 mg/dL). On the fifth day of life he developed signs of disseminated intravascular coagulation [D-dimer 8300 ng/mL (normal: <500), platelets 13,000/μL], and a throat swab specimen was submitted that grew an enterovirus. The isolate was subsequently identified as a CVB3 by antibody neutralization studies and designated CVB3-MCH. Episodes of supraventricular tachycardia developed on the fifth day of life, and he was moved to a pediatric intensive care unit. He developed signs of heart failure and shock on the seventh day of life, and required cardiopulmonary resuscitation. An echocardiogram revealed poor function of both cardiac ventricles. An enterovirus-specific PCR of a blood specimen taken on that day was positive, and a commercially available preparation of intravenous immunoglobulin from pooled donors was given. Serum troponin I measurements were markedly elevated, indicating myocardial injury [maximum 7.3 ng/mL (normal: <0.1 ng/mL)] on the eleventh day of life. His condition remained poor, and his care eventually included extracorporeal membranous oxygenation. Enteroviral RNA was detected again in blood specimens submitted on the 19th and 20th days of life, despite additional infusions of intravenous immunoglobulin. Kidney failure and mild liver dysfunction also developed, and he died on the 36th day of life. Permission was obtained for a limited autopsy, which revealed thickening of the right ventricle and extensive necrosis of the left ventricle. There was also histologic evidence of hemorrhage and necrosis of the kidneys and cholestasis of the liver (not shown). The brain was not examined.

A stock of CVB3-MCH was prepared in Hela-RW cells, and titered by limiting dilution plaque assays, as described previously.[7] To examine the pathogenicity of this isolate, 2 groups of 12-week-old BALB/c mice (10 per group) were infected by intraperitoneal injection with CVB3-MCH or CVB-H3, a molecularly clone of the well-characterized Woodruff strain of CVB3.[8] The CVB3-MCH virus caused death of 6 mice by 6 days post infection; ultimately, 80% of CVB3-MCH infected mice succumbed, compared with 25% mortality among animals infected with CVB-H3. Histologic evidence of myocarditis was found in the animals that died on days 8 and 9 (data not shown).

RNA was extracted from cells infected with CVB3-MCH, and viral sequences were amplified using a Superscript One Step PCR kit (Invitrogen Life Sciences, Carlsbad, CA) with oligonucleotide primers described by Lukashev et al,[6] as well as 2 primers designed specifically to match sequences within the P2 and P3 domains of the viral genome. A complete sequence of the viral genome (GenBank accession no. 1015993) was assembled from these amplicons using the Contig Assembly Program available within BioEdit,[9] and this sequence was aligned using Clustal W with all available full length reference sequences of HEV-B species members. Plots depicting the similarity of CVB3-MCH to other HEV-B members were generated using Simplot, version 3.51 as described by others.[4–6,10] Briefly, similarity was calculated in each window of 500 nucleotides by the Kimura 2-parameter method with a transition-transversion ratio of 2. The window was advanced through the genome alignment in 50 nucleotide steps. As expected from the neutralization data, the sequence constituting the P1 domain of the viral polyprotein, which encodes the viral capsid proteins, clustered with other CVB sequences and was most similar to the sequence from the prototypic CVB3-Nancy isolate (Fig. 1, panels A and C, available online only). In contrast, sequences from the P2 and P3 domains, which encode the nonstructural proteins of picornaviruses), clustered most closely with EV86, a recently identified member of the HEV-B species (Fig. 1, panels B and C, available online only).

To identify potential recombinational relationships of CVB3-MCH with other enteroviruses, an alignment of this isolate with the available prototype HEV-B full length sequences was also analyzed with the bootscanning feature of SimPlot, using a sliding window of 500 bp. Bootscanning analysis revealed evidence that CVB3-MCH is a chimera arising from recombination in the P2 and P3 domains with other HEV-B species, including EV86 and EV97 (Fig. 1, panel D, available online only).

DISCUSSION

Recombination between members of the HEV-B species has been previously described in sequence analyses of isolates from the United States and the former Soviet Union.[4–6] The impact of recombination on the virulence of enteroviruses has not been systematically examined, but this report and 2 previously reported cases of meningitis and acute flaccid paralysis caused by recombinant CVB3[6] demonstrate that recombination does not impair the ability to cause typical disease manifestations, including fatal neonatal disease. Additional examination of the virulence of well characterized and fully sequenced HEV-B recombinants is necessary to clarify the impact of recombination on the pathogenicity of enteroviruses, particularly when this involves newly recognized enteroviruses with poorly described clinical manifestations.[1]

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