CROSS-REACTIVE NEUTRALIZING ANTIBODY AGAINST PANDEMIC 2009 H1N1 INFLUENZA A VIRUS IN INTRAVENOUS IMMUNOGLOBULIN PREPARATIONS

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Abstract: Prepandemic intravenous immunoglobulin (IVIG) and sera from Kawasaki disease patients treated with this IVIG were analyzed for 2009 H1N1-specific microneutralization and hemagglutination inhibition antibodies. All 6 different IVIG preparations tested had significant levels of cross-reactive-specific antibody at a concentration of 2.0 g/dL of immunoglobulin. Sera from 18 of 19 Kawasaki disease patients had significant increases of cross-reactive-specific antibody after 2.0 g/kg of prepandemic IVIG. These results suggest a role for adjunctive IVIG therapy for severe and/or drug-resistant 2009 H1N1 virus and other highly antigenically drifted influenza strains, particularly in the immunocompromised.

Key Words: 2009 H1N1, influenza A, neutralizing antibody, intravenous immunoglobulin, Kawasaki disease

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H1N1 swine-origin influenza A virus (2009 H1N1) initiated a human pandemic in April 2009.[1] Although by the spring of 2010, the activity of this virus declined below pandemic levels in most parts of the world, it is likely to re-emerge during the next seasonal epidemic, as a substantial proportion of the population were not immunized with 2009 H1N1 monovalent vaccine. For example, in the United States, only 40% of children aged between 6 months and 14 years were immunized.[2] Interestingly, early in the pandemic, older individuals appeared to be relatively protected from severe disease from 2009 H1N1, with most infection-associated hospitalizations occurring in young adults (<49 years of age) and children.[3] This contrasts with seasonal influenza epidemics, for which morbidity and mortality are concentrated in the elderly.[4] The relative protection in older adults may be due to pre-existing cross-reactive neutralizing antibody from prior natural infection with H1N1, particularly with 1918 H1N1 and its derivatives,[5] which caused seasonal outbreaks between 1918 and 1957. The formation of protective cross-reactive antibodies from natural infection with H1N1 is plausible, as the hemagglutinin glycoprotein of 2009 H1N1 and the 1918 H1N1 virus and its early derivatives are structurally similar,[6] and hemagglutinin is a well-established target for antibody-mediated protection in humans.[7]

Given the existence of cross-reactive antibodies to 2009 H1N1 in a substantial number of adults, we reasoned that commercial intravenous immunoglobulin (IVIG) products produced prior to the 2009 pandemic, which combine plasma from thousands of adult donors, might contain significant levels of these antibodies. We tested IVIG preparations for cross-reactive microneutralization (MN) and hemagglutination inhibition (HI) antibody against 2009 H1N1, and we also determined whether administration of high-dose IVIG to patients with Kawasaki Disease (KD) patients treated prior to 2009 significantly raised their serum titers of these antibodies.

MATERIALS AND METHODS

Immunoglobulin Preparations. IVIG solutions of 10% (g/dL) immunoglobulin concentration (Gamunex [3 lots], Talecris Biotherapeutics; Gammagard, Baxter Pharmaceuticals; Cytogam and Privigen, CSL Behring), all of which were produced prior to the emergence of 2009 H1N1 in April 2009, were tested. These preparations were diluted in phosphate-buffered saline (PBS) to final concentrations of 1.0, 2.0, and 4.0 g/dL, which encompass the normal IgG concentration in human serum and the peak achievable serum IgG concentration after a 2.0 g/kg dose of IVIG (approximately 3.0 g/dL).[8]

Serum Samples. Sera were obtained from KD patients, aged between 10 months and 10 years, treated in San Diego County, CA from December 2007 to March 2009 with IVIG (Gammagard), following parental informed consent and Institutional Review Board of the University of California at San Diego approval. One group of sera was collected immediately prior to and 1 to 3 days after KD patients received a single 2.0 g/kg dose of IVIG. A second group of sera were collected immediately prior to and 5 to 13 days after other KD patients received two 2.0 g/kg doses of IVIG. Aliquots of sera were frozen at −80°C until thawed for later analysis.

Viral Isolation and Propagation. The 2009 H1N1 influenza A strain, which was obtained from a deidentified primary clinical sample of bronchoalveolar lavage fluid, was grown in Madin-Darby canine kidney cells.[9] This isolate was confirmed as 2009 H1N1 by the California State Department of Public Health using a reverse-transcriptase real-time polymerase chain reaction assay.

HI and MN Assays. HI and MN assays were performed as described previously.[5,10] For the HI assay, serum samples treated with receptor-destroying enzyme (Denka Seiken) were diluted 10-fold (vol/vol) in PBS from which serial 2-fold dilutions were prepared. Diluted sera were mixed with 2009 H1N1 influenza, turkey red blood cells (0.5% vol/vol) were added (Rockland Immunochemicals), and hemagglutination was noted after 30 minutes of incubation. The HI titer was identified as the last dilution that prevented hemagglutination of red blood cells. For the MN assay, heat-inactivated samples were initially diluted 10-fold in PBS and mixed with live 2009 H1N1 influenza. Madin-Darby canine kidney infection was determined by an influenza A nucleoprotein enzyme-linked immunosorbent assay using a mouse anti-nucleoprotein antibody (Millipore). The MN antibody titer was identified as the last serum dilution that prevents infection by 50% as measured by enzyme-linked immunosorbent assay. For calculation of geometric mean titers (GMT), titers were expressed as reciprocal titers (eg, 1:40 was expressed as 40) and an undetectable MN or HI titer was recorded as a reciprocal titer of 5 or one-half the lowest dilution tested.[10] Both the HI and MN assays were validated with negative control serum from an unvaccinated, uninfected volunteer and with positive control serum from an individual who had been infected with H1N1.

Statistical Analysis. Paired, 2-tailed Student *t* test were used to compare (1) MN and HI titers measured in the same IVIG preparation or serum sample and (2) pre- and post-treatment MN or HI antibody titers for sera from the same individual. Pearson *r* values were used to calculate the correlation between MN and HI titers. Prism 5 software (GraphPad) was used for statistical calculations.

RESULTS

Six different commercial preparations of IVIG were tested for the presence of MN and HI titers. All IVIG preparations had detectable levels of MN antibody to 2009 H1N1 at the lowest concentration tested of 1.0 g/dL, which increased in a dose-dependent manner (Fig. 1A). The MN GMT of all preparations was 28.3 at the highest dilution tested of 4.0 g/dL, with some variation between different commercial brands (titers from individual IVIG preparations are presented in Table, Supplemental Digital Content 1, http://links.lww.com/INF/A568). All tested IVIG brands achieved a MN titer of 1:20 at 2.0 g/dL, a concentration that can be readily achieved with the high-doses of IVIG given in KD.[8] Similarly, all IVIG preparations demonstrated dose-dependent increases in HI titers, with a HI GMT of 15.9 at the highest concentration tested of 4.0 g/dL, though there was variation between different lots of the same brand in addition to between brands. There was a significant correlation between the MN titer and HI titer of the IVIG samples at each concentration (Pearson $r = 0.64$, 95% confidence interval = 0.25–0.85, $P = 0.004$), with the MN titer approximately 2-fold higher than the HI titer.

We next determined whether high-dose therapy using prepandemic produced IVIG raised the serum titer of HI and MN antibodies in patients. A single treatment of KD patients with 2.0 g/kg of IVIG increased MN titers against 2009 H1N1 in 18 of 19 subjects (95%) when measured 1 to 3 days postinfusion. Similarly, HI titers also increased in 17/19 (89%) of subjects after 1 dose of IVIG (individual patient titers are presented in Table, Supplemental Digital Content 2, http://links.lww.com/INF/A569). In KD patients who received a single dose of IVIG, both MN and HI titers increased significantly after treatment (Fig. 1B). MN GMT increased from 6.9 to 35.9 ($P < 0.0001$), whereas HI GMT increased from 5.0 to 12.5 ($P < 0.0001$). Again, as in the IVIG preparations, the GMT for MN antibody was approximately 2 times the titer of HI antibody in post-IVIG sera, and this difference was significant ($P < 0.0001$).

In KD patients receiving 2 doses of IVIG, there was also significant increase in MN and HI titers measured within 14 days after the second dose (Fig. 1C). All 8 subjects had increases in the MN titer while 7/8 (88%) of subjects had increases in the HI titer (individual patient titers are presented in Table, Supplemental Digital Content 3, http://links.lww.com/INF/A570). The MN GMT increased from 5.9 to 23.8 ($P = 0.0005$), whereas HA GMTs increased from 5.4 to 13.0 ($P = 0.0038$).

DISCUSSION

Although the 2009 H1N1 influenza A viral pandemic spread rapidly due to the lack of pre-existing immunity in much of the human population, older adults appeared to be relatively protected due to pre-existing cross-protective antibodies that were most likely generated in response to natural infection with 1918 H1N1 and its early derivatives.[5] In this article, we show that commercial preparations of IVIG, produced prior to the 2009 H1N1 pandemic, contain cross-reactive antibodies against 2009 H1N1 as assessed by both HI and MN assays. In addition, we found that administration of high-dose IVIG significantly increased the levels of cross-reactive HI and MN antibodies against 2009 H1N1. Thus,

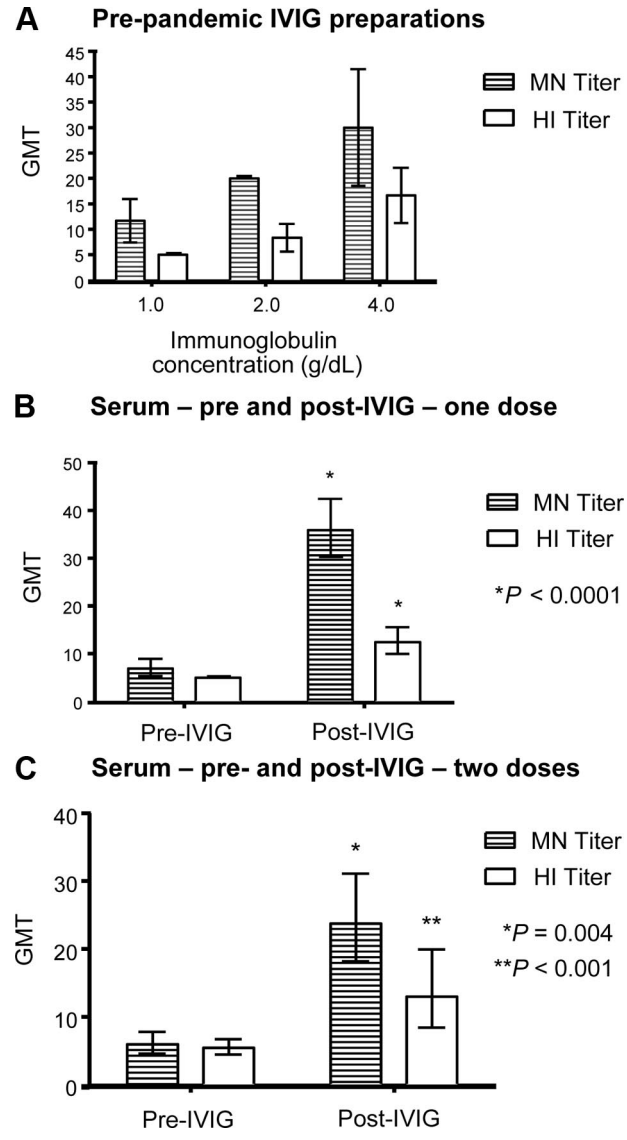

FIGURE 1. A, Microneutralization (MN) and hemagglutination inhibition (HI) antibody titers against 2009 H1N1 in commercial preparations of intravenous immunoglobulin (IVIG). Geometric mean titer (GMT) of MN and HI titers against 2009 H1N1 were determined at 3 different concentrations. Error bars represent 95% confidence interval (CI). B, MN and HI antibody titers against 2009 H1N1 before and after 1 treatment of 2.0 g/kg of IVIG are shown using serum samples that were drawn 1 to 3 days apart. Error bars represent 95% CI. *P* values were determined using the 2-tailed, paired Student *t* test with respect to the pre-IVIG sera sample from the same subject. C, GMT for both MN and HI assays against 2009 H1N1 at baseline, and after 2 treatments of 2.0 g/kg of IVIG, are shown using serum samples that were drawn 5 to 13 days apart following the second dose. Error bars represent 95% CI. *P* values were determined using the 2-tailed, paired Student *t* test with respect to the pre-IVIG sera sample from the same subject.

commercially available IVIG produced prior to the pandemic could potentially be used as an adjunctive treatment for 2009 H1N1 infection in severe cases or in patients with limitations in adaptive immunity. Since newer IVIG preparations will likely include plasma from donors who have been vaccinated for 2009 H1N1 or who were infected with 2009 H1N1, the HI and MN antibody titers will likely increase. However, our results indicate that such inclusion is not necessary for IVIG to provide substantial passive immunity to 2009 H1N1, and suggest that the approach of using existing IVIG preparations might also be considered in future influenza pandemics or if highly drifted strains circulate.

Although there has been no reported clinical experience with the use of passive antibodies in treating 2009 H1N1, there were many attempts to use convalescent blood products in treating acute influenza during the influenza pandemic in 1918. The therapeutic administration of blood products enriched in anti-influenza antibodies appeared to confer a survival advantage particularly if the treatment was given within the first 4 days of illness.[11] In addition, convalescent plasma has been used anecdotally for severe H5N1 infection.[12] Furthermore, in animal models, therapeutic monoclonal anti-influenza IgG antibodies have been shown to clear influenza virus after infection in severe combined immunodeficiency (SCID) mice, which lack B cells and T cells.[13] Thus, it is plausible that the therapeutic administration of immunoglobulin containing neutralizing antibody against 2009 H1N1 could aid in halting the progression of infection as well as in viral clearance.

Passive immunotherapy for 2009 H1N1 influenza and other pandemic influenza A strains may be of particular importance in cases of antiviral resistance. While the 2009 H1N1 virus has remained largely susceptible to the neuraminidase inhibitors, oseltamivir and zanamivir, there have been sporadic reports of oseltamivir-resistant strains. Resistance has been particularly frequent in hosts with impaired adaptive immunity who shed virus for prolonged periods.[14] Adjunctive therapy with IVIG could be particularly useful in this population.

In summary, we have identified significant titers of cross-reactive antibody against 2009 H1N1 in commercial preparations of IVIG despite the low prevalence of pre-existing cross-reactive immunity in the general population. Administration of high-dose IVIG increased the serum titer of these antibodies, and this may be a useful adjunctive therapy in severe infections with 2009 H1N1 influenza, particularly in the immunocompromised, those who are refractory to neuraminidase inhibitor therapy, and immunologically naive children.

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SAFETY AND IMMUNOGENICITY OF A BOOSTER DOSE OF THE 10-VALENT PNEUMOCOCCAL NONTYPEABLE *HAEMOPHILUS INFLUENZAE* PROTEIN D CONJUGATE VACCINE COADMINISTERED WITH DTPW-HBV/HIB AND POLIOVIRUS VACCINES

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Abstract: The safety and reactogenicity profiles of the 10-valent pneumococcal conjugate vaccine, PHiD-CV, and 7vCRM were comparable within the Philippines and Poland when coadministered as a booster dose with DTPw-HBV/Hib and poliovirus vaccines to toddlers primed with the same vaccines. Robust immune responses for all 10 vaccine pneumococcal serotypes and protein D following PHiD-CV booster vaccination were indicative of effective priming.

Key Words: pneumococcal conjugate vaccine, safety, immunogenicity, booster vaccination

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and M.H. declare to have no conflict of interest; S.G., I.D., and L.S. are employed by GlaxoSmithKline Biologicals and have stock ownership; A.F. works as a consultant for GlaxoSmithKline Biologicals.

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The pneumococcal nontypeable *Haemophilus influenzae* (NTHi) protein D conjugate vaccine (PHiD-CV; Synflorix, GlaxoSmithKline Biologicals, Rixensart, Belgium) contains pneumococcal serotypes 1, 5, and 7F in addition to the 7 serotypes contained in the 7-valent pneumococcal conjugate vaccine (7vCRM; Prevenar/Prevnar, Pfizer Inc, New York, NY). A primary vaccination study conducted in the Philippines (6–10–14 weeks schedule, N = 400) and Poland (2–4–6 months schedule, N = 406) assessed the reactogenicity and safety of PHiD-CV as compared with 7vCRM (3:1 ratio) when both were coadministered with the combined diphtheria-tetanus-whole-cell pertussis-hepatitis B/*H. influenzae* type b (DTPw-HBV vaccine, *Tritanrix HepB*, administered in 1 injection with Hib-conjugate vaccine, *Hiberix*) and either oral live attenuated (*Polio Sabin*, the Philippines) or injectable inactivated poliovirus vaccines (IPV, *Poliorix*, Poland).[1] PHiD-CV was immunogenic for each of the 10 pneumococcal vaccine serotypes[1] and was well tolerated, with a reactogenicity and safety profile in line with that of 7vCRM vaccine.[2] In the present study, reactogenicity and immunogenicity were evaluated in both countries after a fourth (booster) dose of PHiD-CV or 7vCRM vaccine coadministered at 12 to 18 months of age with the same vaccines.

MATERIALS AND METHODS

Vaccines were administered intramuscularly into the right (PHiD-CV or 7vCRM), the upper left (DTPw-HBV/Hib), or lower left (IPV) thigh. Study vaccines composition and inclusion/exclusion criteria were as described previously.[1,3]

The primary objective of the study was to demonstrate that a PHiD-CV booster dose was noninferior to 7vCRM in terms of the percentage of subjects reporting fever with rectal temperature >39.0°C after vaccination, on the basis of standardized asymptotic 95% confidence intervals (CIs) for the difference between groups (PHiD-CV minus 7vCRM), and the corresponding 1-sided *P* value for the null hypothesis (H0) that the increase in the percentage of subjects with rectal temperature >39.0°C was above 5% plus half the incidence in the control group.[4] The noninferiority objective was reached if H0 could be rejected with *P* value <0.025.

Assessments of reactogenicity and safety were as described for previous PHiD-CV booster trials.[2] Specific local and general adverse events (AEs) were solicited for 4 days after vaccination and scored on a 3-grade intensity scale, and subjects were monitored up to 31 days after vaccination for unsolicited events. Large swelling reactions were also recorded. Serious adverse events (SAEs) were monitored throughout the entire study period. Immunogenicity was assessed in blood samples taken before (day 0) and 1 month after the booster dose, as described previously.[1,5] In a subset of 200 subjects from each country, serum antipneumococcal IgG concentrations were measured using GlaxoSmithKline's 22F-inhibition enzyme-linked immunosorbent assay (ELISA), with a reference antibody concentration of 0.2 μg/mL, opsonophagocytic activity (OPA) by a killing assay, with a cut-off opsonic titer of 8, and antibodies against NTHi protein D by ELISA. ELISA geometric mean antibody concentrations (GMCs) and/or OPA geometric mean titers (GMTs) were calculated for each vaccine serotype, protein D, and cross-reactive serotypes 6A and 19A. Antibodies to the coadministered vaccine antigens were also analyzed (Text and Table, Supplemental Digital Content 1, http://links.lww.com/INF/A576).

RESULTS

Of the 756 children enrolled and included in the total vaccinated cohort (383 in Poland and 373 in the Philippines), 12 (all from Poland; 10 in PHiD-CV group, 2 in 7vCRM group) did not complete the booster study because of consent withdrawal and not due to an AE, protocol violation, or move from study area. A total of 703 were included in the according-to-protocol cohort for immunogenicity (Fig., Supplemental Digital Content 2, http://links.lww.com/INF/A577). In each country, demographic characteristics of the PHiD-CV and 7vCRM groups were comparable (Table, Supplemental Digital Content 3, http://links.lww.com/INF/A578).

The primary objective of noninferiority in terms of the occurrence of rectal temperature >39.0°C following PHiD-CV booster vaccination in both countries (11.5%) compared with 7vCRM booster vaccination (10.6%) was reached since the upper limit of the 95% CI for the difference between groups (0.89% [95% CI: −4.82%–5.59%]; $P < 0.001$) was lower than 10.3%. Opposite trends in incidences of postvaccination febrile reactions (rectal temperature >39°C) were however observed in the individual countries, with a trend for higher fever incidence in 7vCRM versus PHiD-CV recipients in the Philippines and an inverse trend in Poland (Table 1). Only 1 case of grade 3 fever (rectal temperature >40.0°C) was reported (PHiD-CV group, Poland).

In each country, the incidence of each solicited AE (of any intensity) was in the same range for both groups (overlapping 95% CIs) (Table 1). However, incidences of solicited AEs tended to be higher in Poland than in the Philippines (Table 1), including high (≥40.6%) incidences of grade 3 pain in both groups in Poland. Incidences of grade 3 solicited general AEs were low (maximum 5.2%) in both countries, except for irritability (14.4%) in PHiD-CV recipients in Poland (Table 1).

Large swelling reactions were reported in 5 subjects in the Philippines (4 at DTPw-HBV/Hib, 1 at PHiD-CV injection site) and 14 subjects in Poland (10 at DTPw-HBV/Hib, 3 at PHiD-CV, 1 at IPV injection site). All were local or diffuse swelling reactions not involving adjacent joints and resolved without sequelae. The incidence of unsolicited AEs was lower in the Philippines (8.9%, PHiD-CV vs. 9.7%, 7vCRM) than in Poland (37.2% vs. 35.7%). SAEs were reported in 2 subjects in the Philippines (2/280 children, PHiD-CV) and 7 subjects in Poland (5/285, PHiD-CV; 2/98, 7vCRM). One SAE (fever with pharyngitis that started on day of PHiD-CV vaccination in Poland) was assessed to be causally related to vaccination. No fatal SAEs were reported up to the 1 month follow-up visit. During an extended safety follow-up period (up to approximately 6 months after booster vaccination), 1 SAE reported in the Philippines had a fatal outcome; persistent vomiting started 33 days after the 7vCRM booster dose and resulted in hospitalization 1 month later, after which intracranial mass was diagnosed, complicated with acute respiratory distress and death 1 week later. The fatal SAE was not considered to be causally related to vaccination.

For each of the pneumococcal serotypes common to both vaccines, robust immune responses were observed 1 month after the booster dose in both groups in each country. The range of pre-

TABLE 1. Incidences of Solicited Local (Any Injection Site) and General Adverse Events (% [95% CI]) Within 4 Days (Days 0–3) After Booster Vaccination (Total Vaccinated Cohort)

Symptom	Intensity*	Philippines		Poland	
		PHiD-CV (N = 280)	7vCRM (N = 93)	PHiD-CV (N = 278)	7vCRM (N = 96)
Pain	Any	72.5 (66.9–77.6)	71.0 (60.6–79.9)	89.2 (85.0–92.6)	80.2 (70.8–87.6)
	Grade 3	14.3 (10.4–18.9)	21.5 (13.7–31.2)	42.1 (36.2–48.1)	40.6 (30.7–51.1)
Redness	Any	38.2 (32.5–44.2)	39.8 (29.8–50.5)	70.9 (65.1–76.1)	68.8 (58.5–77.8)
	>30 mm	2.9 (1.2–5.6)	3.2 (0.7–9.1)	12.6 (8.9–17.1)	11.5 (5.9–19.6)
Swelling	Any	32.9 (27.4–38.7)	34.4 (24.9–45.0)	56.8 (50.8–62.7)	54.2 (43.7–64.4)
	>30 mm	7.5 (4.7–11.2)	9.7 (4.5–17.6)	12.2 (8.6–16.7)	8.3 (3.7–15.8)
Irritability	Any	61.8 (55.8–67.5)	68.8 (58.4–78.0)	87.4 (82.9–91.1)	82.3 (73.2–89.3)
	Grade 3	4.3 (2.2–7.4)	3.2 (0.7–9.1)	14.4 (10.5–19.1)	5.2 (1.7–11.7)
Drowsiness	Any	32.1 (26.7–38.0)	34.4 (24.9–45.0)	68.3 (62.5–73.8)	68.8 (58.5–77.8)
	Grade 3	1.8 (0.6–4.1)	1.1 (0.0–5.8)	2.5 (1.0–5.1)	2.1 (0.3–7.3)
Loss of appetite	Any	34.6 (29.1–40.5)	30.1 (21.0–40.5)	66.2 (60.3–71.7)	59.4 (48.9–69.3)
	Grade 3	2.9 (1.2–5.6)	1.1 (0.0–5.8)	4.7 (2.5–7.9)	1.0 (0.0–5.7)
Fever (rectal temperature)	≥38.0°C	49.3 (43.3–55.3)	54.8 (44.2–65.2)	77.3 (72.0–82.1)	67.7 (57.4–76.9)
	>38.5°C	19.3 (14.8–24.4)	20.4 (12.8–30.1)	46.0 (40.1–52.1)	36.5 (26.9–46.9)
	>39.0°C	4.3 (2.2–7.4)	8.6 (3.8–16.2)	18.7 (14.3–23.8)	12.5 (6.6–20.8)
	>39.5°C	1.1 (0.2–3.1)	3.2 (0.7–9.1)	7.6 (4.7–11.3)	3.1 (0.6–8.9)
	>40.0°C	0.0 (0.0–1.3)	0.0 (0.0–3.9)	0.4 (0.0–2.0)	0.0 (0.0–3.8)

*Adverse event of grade 3 intensity: pain, crying when limb was moved or limb was spontaneously painful; irritability, crying that could not be comforted/prevented normal everyday activity; drowsiness, prevented normal everyday activity; loss of appetite, child did not eat at all.

N indicates number of subjects with documented dose; PHiD-CV, protein D conjugate vaccine; 7vCRM, 7-valent pneumococcal conjugate vaccine.

to postbooster fold increases in antibody GMC was 10.7 to 27.6 in PHiD-CV and 15.0 to 41.0 in 7vCRM vaccinees in the Philippines, and 7.0 to 16.6 in PHiD-CV and 14.5 to 28.0 in 7vCRM vaccinees in Poland. In the Philippines, postbooster antibody GMCs were significantly higher in PHiD-CV than in 7vCRM recipients for antibodies against serotypes 18C and 19F, and lower for serotypes 14 and 9V, whereas in Poland GMCs were significantly higher in the PHiD-CV group for serotype 19F and lower for serotypes 4, 6B, 9V, 14, and 23F (Table, Supplemental Digital Content 4, http://links.lww.com/INF/A579). In both countries, percentages of subjects with postbooster antibody concentrations ≥0.2 μg/mL were at least 95.3% for each of the 7 common serotypes and within the same range between groups.

OPA seropositivity rates in the Philippines were at least 92.9% and in the same range for all 7 common serotypes except 19F (tended to be lower in 7vCRM group), and OPA GMTs were in the same range, except for serotypes 18C and 19F (higher in PHiD-CV group) and 6B, 9V, and 23F (higher in 7vCRM group) (Table, Supplemental Digital Content 5, http://links.lww.com/INF/A580). In Poland, although OPA GMTs were higher in the 7vCRM group for the 7 common serotypes, except serotypes 19F (higher in PHiD-CV group) and 18C (within same range), OPA seropositivity rates (at least 88.3%) were within the same range in both groups (Table, Supplemental Digital Content 5, http://links.lww.com/INF/A580). For each of the additional serotypes 1, 5, and 7F, in both PHiD-CV groups, at least 99.2% of subjects had antibody concentrations ≥0.2 μg/mL and at least 95.0% had OPA titers ≥8.

For cross-reactive serotype 6A, at least 79.2% of subjects had antibody concentrations ≥0.2 μg/mL or OPA titers ≥8 after PHiD-CV or 7vCRM booster vaccination in both countries (Tables, Supplemental Digital Content 4 and 5, http://links.lww.com/INF/A579 and http://links.lww.com/INF/A580, respectively). Percentages for cross-reactive serotype 19A were lower in 7vCRM than in PHiD-CV recipients. Antiprotein D antibody GMCs increased to 2769.6 EL.U/mL in Poland and 4973.9 EL.U/mL in the Philippines following PHiD-CV booster vaccination, compared with 91.6 and 124.1 EL.U/mL, respectively, following 7vCRM booster vaccination.

DISCUSSION

This study in Filipino and Polish children showed that a PHiD-CV booster dose had a safety and reactogenicity profile that was in line with that of a 7vCRM booster dose, when both vaccines were coadministered with DTPw-HBV/Hib and poliovirus vaccines. Also, for the 7 pneumococcal serotypes common to both vaccines, the percentages of subjects reaching ELISA and OPA thresholds were within the same range after PHiD-CV and 7vCRM booster vaccination.

Within the same country, incidences of AEs were in the same range for the PHiD-CV and 7vCRM groups, apart from a trend for a higher incidence of fever in the PHiD-CV group in comparison with the 7vCRM group in Poland. This trend was not observed in the Philippines, where incidences of fever were within the same range or (for overall fever and fever >39.0°C) tended to be lower in PHiD-CV recipients. With DTPw-based vaccines, incidences of AEs tend to increase with age at priming and the number of injections,[6] so it is possible that higher reactogenicity in Polish children was at least partly associated with the later age for primary vaccination in this group, although it is not clear whether this effect persists with the booster dose.

Robust increases in ELISA antibody concentrations and OPA titers after booster vaccination for all pneumococcal vaccine serotype-specific antibodies and protein D indicate effective priming of the immune system. Moreover, for the 7 serotypes common to PHiD-CV and 7vCRM, the proportions of children reaching the 22F-ELISA threshold or OPA cut-off were high after booster vaccination with both vaccines. As found after primary vaccination,[1] generally higher immune responses were observed in the Philippines compared with the Polish groups, and antibody GMCs and OPA GMTs in the Philippines were higher than in previous European PHiD-CV booster studies.[7–9] We reported previously that this could not be accounted for by differences in Bacillus Calmette-Guérin vaccine administration at birth,[1] and this was confirmed on the basis of the postbooster results (data not shown).

In Poland, ELISA GMCs and OPA GMTs for the 7 pneumococcal serotypes in common were usually lower in the PHiD-CV

group than in the 7vCRM group. In contrast, in the Philippines, immune responses in the PHiD-CV group were within the same range for most of the 7 serotypes and higher for serotypes 18C and 19F, which was consistent with the primary study results.[1]

In conclusion, the safety and reactogenicity profiles of PHiD-CV and 7vCRM were comparable when used for booster vaccination coadministered with DTPw-HBV/Hib and poliovirus vaccines in the second year of life. Robust immune responses for all 10 vaccine pneumococcal serotypes and NTHi protein D following PHiD-CV booster vaccination in both the Philippines and Poland are indicative of effective priming with the 2 different primary vaccination schedules and therefore lend further support to the protective effect of PHiD-CV against vaccine serotype pneumococcal infections.

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LATE PREGNANCY SCREENING FOR HUMAN IMMUNODEFICIENCY VIRUS

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Abstract: Three cases are presented of infants born to mothers who were screened and tested negative for HIV during their first trimesters. None of these pregnant women were retested late in their pregnancies, as they were not considered at high risk for HIV infection. All were found to be infected shortly after giving birth. Of the 3 infants, 2 developed HIV infection. These cases support a recommendation for late pregnancy HIV screening of all women.

Key Words: HIV screening, pediatric AIDS, pediatric HIV, HIV prevention, pregnancy screening

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The Centers for Disease Control and Prevention (CDC) and American Academy of Pediatrics (AAP) currently recommend routine HIV-1 testing of all pregnant women. In the current CDC guidelines[1] it is stated that testing should be performed "as early during pregnancy as possible," whereas the 2009 Red Book[2] does not indicate timing for testing. These 2 sources are used by most obstetricians, pediatricians, and primary care physicians who care for pregnant women and their newborn infants. Neither of these documents clearly recommends repeat testing late in pregnancy regardless of maternal risk factors.

The CDC subsequently revised their guidelines for HIV testing of pregnant women to recommend that a second screening enzyme immunoassay (EIA) for HIV antibody be performed late in pregnancy, but <36 weeks gestation for women with high-risk behavior and those living in 20 HIV high-incidence states (AL, CT, DE, FL, GA, IL, LA, MD, MA, MS, NV, NJ, NY, NC, PA, RI, SC, TN, TX, and VA), the District of Columbia, and Puerto Rico.[3] Also included are those pregnant women who receive their health care in facilities where the incidence of HIV infection is >1/1000 women screened.

The present report describes 3 neonates born to mothers who were tested for HIV early in their pregnancies, did not fulfill risk categories for late pregnancy testing according to current guidelines, and were reported negative, but who acquired infection later in their pregnancies. All 3 mothers lived in New Orleans, LA, and delivered at private hospitals in the city. Two of their children were subsequently diagnosed with HIV infection. A recommendation for testing both early and late in pregnancy for all pregnant women is supported by these cases.

CASE REPORTS

Case 1. A 4-month-old female infant was admitted from the emergency department with respiratory failure requiring intubation. She was afebrile and had extensive bilateral pneumonia.

Review of maternal history documented that the mother was screened for HIV during her first trimester and was negative. She had denied injection drug use. It was not known that she had a new conjugal partner during her pregnancy. She did live in Louisiana which is considered a high-risk state, but was followed up and delivered at a medical facility where the incidence of HIV infection is well less than 1/1000 women screened. Late HIV testing was not considered necessary, and so was not performed. The mother breast-fed her infant until the time of her infant's illness and hospitalization.

Evaluation of the infant revealed that she was HIV-positive with a viral load of 130,755 copies/mL. The cause of her pneu-

monia was cytomegalovirus, documented with a blood PCR. Her cytomegalovirus was treated with ganciclovir and she was started on HAART therapy with lopinavir/ritonavir, zidovudine, and lamivudine. Her mother was evaluated and shown to be HIV-positive with a viral load of 19,502 copies/mL.

Case 2. A female infant was delivered to a mother whose prenatal care was given outside of Louisiana in a state considered low risk for HIV infection. The mother recently moved to New Orleans and had established contact with a local obstetrician but had not yet been seen. She presented to the emergency department in labor with a history that she had been screened for HIV and was negative. This could not be confirmed. When screening tests for syphilis, chlamydia, and hepatitis B, surface antigen were all positive; an EIA for HIV was performed and was positive. The neonate was started on zidovudine therapy on day 3 of life but was subsequently positive for HIV by PCR-DNA at birth and 1 month of age and was started on HAART therapy.

Case 3. A 6-week-old male infant was referred when the child's father had recently been diagnosed with acute HIV infection, manifest by persistent fever, night sweats, lymphadenopathy, and a 20-pound weight loss. The infant's mother had been screened early in her pregnancy, was negative, and was not considered to have any risk factors for HIV disease. When her husband was diagnosed with AIDS, she was tested and was positive. She had been breast-feeding her infant. HIV-PCR DNA on the infant was negative at the time of referral, at 2 months, and at 4 months of age. Follow-up indicates that the infant is not infected.

DISCUSSION

A marked reduction in mother-to-child transmission (MTCT) of human immunodeficiency virus type 1 followed publication of the clinical trial of maternal zidovudine treatment (protocol 076) in 1994[4] and recommendations for its routine application published by CDC the same year.[5]A further decrease in vertical transmission is attributed to 2 additional interventions: avoidance of breast-feeding and cesarean delivery before onset of labor and before rupture of membranes. More recent studies have shown that highly active antiretroviral therapy given to HIV-infected mothers can reduce MTCT of HIV to just 1%.[6] However, the obvious first step in assuring these preventive interventions is identification of all HIV-positive mothers.

A statement published by the Committee on Pediatric AIDS in 2008 suggested, but did not clearly recommend, repeat testing in the third trimester or rapid testing at labor and delivery to further reduce the rate of perinatal HIV transmission.[7] The committee pointed out that testing late in pregnancy or even after delivery of the newborn infant allowed beneficial interventions that have been shown to reduce HIV infection in exposed neonates. Even when mothers are not identified as HIV infected until onset of labor, treatment with intravenous zidovudine along with treatment of the neonate with oral zidovudine for 6 weeks may reduce the likelihood of infection in infants by as much as 60%.[7,8] This benefit may be lower as the original study[8] demonstrating reduction used an observational cohort format, and there was a wide confidence interval because of the low number of HIV infected neonates.

An additional factor to consider in making a decision for routine repeat testing of all pregnant women is the high levels of viral replication with acute HIV infection that significantly increase the likelihood of vertical transmission for mothers who acquire disease during their pregnancies.[7]

The only suggested downside to routine rescreening late in pregnancy is cost. However, under most current circumstances, a second HIV test during the third trimester has been shown to be cost-effective when the HIV incidence is 1.0 per 1000 person-years or higher.[9] Moreover, studies have shown that rescreening with the rapid HIV test in the third trimester of pregnancy is well-accepted by both low and high risk mothers and can direct treatment that is effective in preventing perinatal HIV transmission.[10]

Current management of HIV-infected women identified late in their pregnancies is essentially the same as that for women diagnosed earlier, that is, 3 antiretroviral drugs for prophylaxis continued to the time of delivery. Intravenous zidovudine is then administered to the pregnant woman at the time of labor and continued until the cord is clamped, while the other anti-retroviral components of the regimen are continued orally during labor. Oral zidovudine is then administered to the infant for the first 6 weeks of life. For women diagnosed late, particularly if this is not accomplished until they are in labor, consideration might be given to the administration of nevirapine intrapartum and to the neonate to further reduce the likelihood of MTCT.[11]

The present cases support a recommendation for routine rescreening of pregnant women late in pregnancy, ie, at 36 to 37 weeks gestation or at the time of labor and delivery. However, unless this becomes an official recommendation from the American Academy of Pediatrics or the Centers for Disease Control and Prevention, insurance companies will not reimburse for such laboratory testing. At present, few health insurance policies include payment for a second HIV screen. This is our current situation, a major limitation for its routine implementation in most hospitals.

Our case 1 brings up another issue related to routine child care. This infant might have become infected from the mother's breast milk, if the mother had not acquired her disease until after she delivered. Such a situation suggests that pediatricians should include a detailed maternal social history during routine visits of breast-fed infants. High-risk maternal behavior or known disease in their partners warrants repeat testing of these mothers for HIV infection while they are breast-feeding.

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NASAL *STAPHYLOCOCCUS AUREUS* COLONIZATION AMONG MOTHERS OF TERM AND LATE PRETERM PREVIOUSLY HEALTHY NEONATES WITH COMMUNITY-ACQUIRED *STAPHYLOCOCCUS AUREUS* INFECTIONS

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Abstract: We enrolled 35 case neonates with community-acquired *Staphylococcus aureus* infection and their mothers and 19 control mother-neonate pairs. We obtained neonatal and maternal anterior nasal cultures, and clinical isolates. *S. aureus* nasal colonization was greater in case than control pairs. Neonates were more often infected with their nasal strain than their mother's nasal strain.

Key Words: *Staphylococcus aureus*, community-acquired infections, colonization, infant, mother

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Methicillin resistant *Staphylococcus aureus* (MRSA) infections are increasing in previously healthy neonates without traditional risk factors.[1,2] At Texas Children's Hospital (TCH), we noted a peak of community-acquired *S. aureus* infections at 7 to 12 days after delivery in both mothers and neonates, suggesting the acquisition of *S. aureus* from the mother during or after delivery, birth hospital, home, or from family members.

Our primary hypotheses were (1) previously healthy term and late preterm neonates with *S. aureus* infections and their mothers were more likely to have nasal colonization with *S. aureus* than control subjects and their mothers and (2) the microbiologic and molecular characteristics of *S. aureus* clinical isolates will be identical to those of the *S. aureus* nasal isolates of the neonates and their mothers.

PATIENTS AND METHODS

The Baylor College of Medicine Institutional Review Board approved this study. From September 2005 to May 2007, after the parent's consent, we enrolled previously healthy neonates with suspected *S. aureus* infections or fever without localizing source (control subjects) and their mother during the first 24 hours of treatment. We enrolled additional mother-neonate pairs when a previously unsuspected *S. aureus* infection was diagnosed by culture within 48 hours of TCH admission. Cases were neonates with a culture-confirmed *S. aureus* infection. Controls included neonates initially suspected to have *S. aureus* infection but subsequently diagnosed with an infection caused by another organism.

Previously healthy neonates were 36 weeks gestation or greater at birth and 30 days of age or less at TCH admission as defined previously.[1,2] After a detailed medical history using a standardized interview instrument, maternal and neonatal anterior nasal cultures were obtained.

Nasal swabs were inoculated onto Columbia agar with 5% sheep blood, colistin, and nalidixic acid directly and after the swab was agitated in 100 uL normal saline. One colony with typical *S. aureus* morphologic features was subcultured on to a second TSA sheep blood agar plate. *S. aureus* was identified by the presence of coagulase, using a latex agglutination test (StaphTEX Kit, Hardy Diagnostics, Santa Maria, CA). *S. aureus* strains isolated from clinical specimens were collected from the TCH microbiology laboratory. Antibiotic susceptibility was determined by disk diffusion or broth dilution, using Clinical and Laboratory Standards Institute guidelines.[3]

S. aureus pulsed-field gel electrophoresis was performed and interstrain relationships were determined as described previously.[4] Banding patterns (pulsotypes) of the mother's and the neonate's paired isolates had to be identical to be considered the same.

Statistical analysis was performed by using Fisher exact test or χ^2 for dichotomous variables or Student *t* test for comparison of means by using VassarStats: Web Site for Statistical Computation.[5] Analyses were 2-tailed, and a P value of ≤ 0.05 was considered significant.

RESULTS

We enrolled 60 mother-neonate pairs with suspected neonatal *S. aureus* infection and 11 pairs with neonatal fever (control subjects). After final culture results, subjects were classified as 35 case and 19 control mother-neonate pairs. Non *S. aureus* pathogens were isolated from 8 other infected neonates subsequently classified as controls. Infections of subjects enrolled after the second hospital day included bacteremia, empyema, omphalitis, sacral abscess, and lacrimal duct inflammation (a control subject).

More case (25/35 [71%]) than control neonates (4/19 [21%]) had *S. aureus* nasal colonization ($P < 0.001$). More case (12/35 [34%]) than control mothers (1/19 [5%]) had *S. aureus* nasal colonization ($P = 0.02$). However, the proportion of case and control mother-neonate pairs with identical nasal colonizing isolate pulsotypes (6/35 [17%] vs. 0/19 [0%]) did not differ significantly.

Identical maternal nasal and neonatal clinical isolate pulsotypes were found in 8/34 (24%) case mother-neonate pairs. Identical neonatal nasal and neonatal clinical isolate pulsotypes were found in 21/34 (62%) case mother-neonate pairs. Identical maternal nasal, neonatal nasal, and neonatal clinical isolate pulsotypes were found in 6/35 (17%) of case mother-neonate pairs.

USA300 isolates were not found in control mother-neonate pairs (Table 1). Most MRSA isolates (46/48 [96%]) and 5 methicillin-susceptible *S. aureus* (MSSA) isolates were USA300. Two MRSA isolates were USA400 and a unique pulsotype. The other MSSA isolates were 2 USA700 pulsotypes, 3 USA400 pulsotypes, and 29 unique pulsotypes; 2 were related to USA300. In 4 mother-infant pairs, isolate pulsotypes differed by only 1 or 2 bands (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A619).

Infants were born at 27 different hospitals and 1 at home. After excluding USA300, only 1 large nearby hospital had 2 neonates, born 1 month apart, affected by the same unique MSSA pulsotype.

The characteristics and medical histories of case and control subjects were not significantly different except for racial distribution and male circumcision (Table, Supplemental Digital Content

TABLE 1. Distribution of Pulsed-field Gel Electrophoresis Patterns for Case and Control Mother-infant Pairs

	Case Subjects			Control Subjects	
	Clinical	Neonate Nasal	Mother Nasal	Neonate Nasal	Mother Nasal
USA300					
MRSA	22	16	6	—	—
MSSA	2	1	2	—	—
Other					
MRSA	—	—	—	2	—
MSSA	8	8	4	2	1
Unknown					
MRSA	3	—	—	—	—

— indicates no isolates; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

2, http://links.lww.com/INF/A618). Case subjects were more often white and control subjects were more often Hispanic ($P = 0.01$). Male case subjects were more likely to be circumcised than male control subjects ($P = 0.001$).

A current or past skin or soft-tissue infection history was present more often for case mothers ($P = 0.01$) and case families ($P = 0.04$) than control mothers or families. All MRSA colonized mothers had a maternal or family history of skin or soft-tissue infection, which was significantly greater than mothers not colonized by *S. aureus* or mothers colonized by MSSA ($P = 0.003$, $P = 0.01$, respectively). Mothers colonized with MRSA were more likely to have received antibiotics during or after delivery compared with mothers not colonized ($P = 0.02$, $P = 0.04$, respectively).

DISCUSSION

We describe the first study to evaluate the association of nasal colonization status and other infection risk factors in previously healthy neonates infected with *S. aureus* and their mothers compared with control subjects and their mothers. Increasing *S. aureus* infections in previously healthy neonates underscores the importance of understanding the source of neonatal *S. aureus* acquisition and infectious risk factors.[1,2] We found a significant relationship between nasal colonization of the mother and neonate with neonatal infection. Nasal colonization was greater in previously healthy term and late preterm neonates with *S. aureus* infections and their mothers than in control neonates and mothers. However, the strain causing the infection in neonates was not recovered from the anterior nares of their mothers 76% of the time.

One-third of the case mothers were nasally colonized with *S. aureus*. Two-thirds of these mothers had identical nasal strains by pulsotype and antimicrobial susceptibilities to their infected neonates' clinical isolate. In some nonidentical cases, strain pulsotypes differed by only 1 or 2 bands. The minimally different isolates within one family may reflect simultaneous acquisition of similar but nonidentical bacteria from a shared environment or genome modifications after transmission between the mother and child.

Most MRSA isolates were USA300 pulsotypes and most MSSA isolates were non-USA300 pulsotypes, consistent with our studies of community infections.[1,6] Case neonates and mothers were more likely to carry USA300 nasally than control neonates and mothers. In fact, USA300 isolates were not found in any control neonates or their mothers.

Potential infection risk factors identified by this study include ethnicity, circumcision, family and maternal skin, and soft-tissue infection history, and maternal antibiotic use. Identification of families with infection history should prompt diligent observation of the mother and neonate for infectious symptoms. Case families were more often white, and control families were more often Hispanic. The case family ethnicity was similar to our previously reported population.[1] The greater circumcision rate in case families may reflect an entry portal for the organism or the enrolled subjects' ethnicity. Our prior observational study with a larger sample size did not reveal any association with circumcision.[1]

We only investigated one of many possible sources of acquisition. Other sources could include: (1) the delivery hospital, although many hospitals were affected in this study, (2) other family members, (3) simultaneous maternal and infant acquisition of the flora, or (4) maternal colonization at another site including throat, skin, breast milk, or vaginal flora.[7]

Prevention of neonatal *S. aureus* infection is the ultimate goal. If mothers are the primary reservoir, identifying the carriage site and mode of transmission is crucial.[8] *S. aureus* colonization of healthy neonates within the first 2 weeks of life has correlated with maternal *S. aureus* colonization in some studies,[9] but not in others.[10]

The strengths of our study include the assessment for infection risk factors, the evaluation of mothers and neonates, and direct family interviews. Limitations of our study are the retrospective assessment for infection risk factors, assessment for colonization at only 1 site, and fewer enrolled control families than case families related, in part, to the reluctance of some families to have their neonate participate without a suspected *S. aureus* infection. Our sensitivity for detection of *S. aureus* nasal colonization may not have been optimal, as we did not subculture the nasal swabs in broth. However, many investigators use this method, and all cultures were handled the same way.

In conclusion, nasal colonization was more likely in previously healthy term and late preterm neonates with *S. aureus* infections and their mothers than in control subjects and their mothers. Neonates were more often infected with their own nasal strain than with their mother's nasal strain. Maternal nasal colonizing strains are likely not the sole source of neonatal nasal colonization or infection.

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STREPTOCOCCUS PNEUMONIAE NASOPHARYNGEAL COLONIZATION IN CHILDREN IN BRASOV, CENTRAL ROMANIA

HIGH ANTIBIOTIC RESISTANCE AND COVERAGE BY CONJUGATE VACCINES

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Abstract: We report high colonization rates among 400 healthy infants and children, and moderate (66%) coverage by PCV7 and PCV10, with a superior (80%) PCV13 coverage. Most frequent serotypes were 23F, 6B, 19F, and 14. Resistance to penicillin, ceftriaxone, erythromycin, and trimethoprim/sulfamethoxazole was 83%, 18%, 62%, and 66%, respectively. 67% isolates were multidrug resistant. Pneumococcal conjugate vaccines covered 80% to 93% of multidrug resistant isolates.

Key Words: *Streptococcus pneumoniae*, nasopharyngeal colonization, children, Romania, conjugate vaccines

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Acquisition of resistance of *Streptococcus pneumoniae* to antimicrobial agents was sporadically reported in Romania during the last 2 decades. In a multinational study, 41% of the 82 *S. pneumoniae* isolates obtained from Romanian children with acute otitis media (AOM) were intermediately or fully resistant to penicillin.[1] An additional study, investigating pneumococcal nasopharyngeal (NP) colonization in HIV-negative and HIV-positive children living in an orphanage in Northeastern Romania, revealed high multidrug resistant (MDR) rates, high rates of recovery of serotypes 19A and 23F, and the presence of unique Romanian *S. pneumoniae* clones.[2,3]

The aims of this study was to analyze the antimicrobial susceptibility and serotype distribution of NP *S. pneumoniae* isolated from 4 groups of infants and children (attending DCCs, community immunization clinics, emergency room [ER], and surgery department) in the city of Brasov, Central Romania.

PATIENTS AND METHODS

Study Population. Brasov has a population of ~400,000; the Children's Hospital is the only pediatric hospital in the city. Starting April 1, 2008 and ending January 31, 2009, NP cultures for *S. pneumoniae* were performed in: (1) 100 healthy children, 2 to 5 years old, attending 6 DCCs in the city. The great majority of children >2 years of age in Romania attend DCCs while all children <2 years stay at home until the DCC age. Each DCC was visited only once for samples collection; (2) 100 consecutive healthy children <5 years old visiting the clinics of 5 community pediatricians for completion of immunizations; (3) 100 consecutive healthy children <5 years old examined at ER with trauma or noninfectious diseases; (4) 100 consecutive healthy children <5 years old hospitalized for elective surgical procedures. The research protocol was approved by the Ethics Committee of the hospital.

Laboratory Procedures (Sampling and Identification). The NP specimens were collected by swabs in MW173Amies transport medium (Transwab; Medical Wire and Equipment) and cultured at the Microbiology Laboratories of the hospital within 24 hours as previously described.[4] The identified organisms were subcultured and stored at −70°C. The stored organisms were further transported by air to Israel for organism confirmation, determination of antimicrobial susceptibility and serotyping. All 52 serotype 23F isolates were also analyzed by disk susceptibility to ciprofloxacin, ofloxacin, levofloxacin, and moxifloxacin.

RESULTS

Overall, 400 infants and young children <5 years old were enrolled, 100 at each of the 4 sampling centers. Mean age was 31.4 ± 19 months; the youngest patients were those visiting the immunization clinics (mean age: 9.4 ± 8.2 months) and the oldest those attending DCCs (42.5 ± 13.2 months). One hundred sixty-two (40.5%) were <2 years old. Sixty (15%) children received antibiotics during the 48 hours preceding enrollment and 97 (24%) during the previous month. Data on the specific antibiotics received were available in 82/97(85%) patients. The most commonly used antibiotics were amoxicillin (20/82 patients, 24%), amoxicillin/clavulanate (19, 23%), and clarithromycin (13, 16%).

Total *S. pneumoniae* colonization rate was 51% (205/400 patients) (Table 1). The highest colonization rates were recorded in DCCs (71%) and the lowest at ER (34%), $P < 0.001$. The colonization rates among children <12 months and 13 to 24 months were 59% and 73%, respectively ($P = 0.1$). Of the 92 patients who received antibiotics during the previous month, 53 (58%) had positive NP cultures. The most frequently isolated serotypes among these patients were 23F (20 isolates, 38%), 19F (6, 11%), 6B (6, 11%), and 14 (3, 6%); 12/53 (23%) and 37/53 (70%) were susceptible to penicillin and ceftriaxone, respectively; 34 (64%) isolates were MDR.

Twenty-four serotypes were identified; the most frequently isolated were 23F, 6B, 19F, and 14 (25%, 15%, 13%, and 10%, respectively, of all isolates). The PCV7-associated serotypes 6A and 19A represented 8% and 5%, respectively, of all isolates; 9/11 (82%) of serotype 19A isolates were recovered at the DCC. The distribution of the other main serotypes was similar between the 4 sampling centers and between the patient age groups. Serotypes 11A (6 isolates, 3% of all isolates) and 15 B/C (4, 2%) were the most commonly isolated nonvaccine serotypes.

The serotypes included in PCV7 represented 66% (135/205) of all isolates. By including the closely related serotype 6A (8% of all isolates), PCV7 and PCV10 each covered 74% of all isolates; 164/205 (80%) of the isolates were included in the PCV13, with no differences between the isolates recovered at each of the sampling centers.

Eighty-three percent and 18% of all isolates were nonsusceptible to penicillin and ceftriaxone, respectively; 40.5% and 16% of all isolates were highly-resistant to penicillin and ceftriaxone. The nonsusceptibility percentages among the *S. pneumoniae* isolates were similar among the isolates recovered at each of the 4 sampling centers.

TABLE 1. Colonization Data on Various Age Group Among 400 Infants and Children Enrolled at 4 Sampling Centers

Age (mo)	Daycare Center (n = 100)	Elective Surgery (n = 100)	Immunization Clinics (n = 100)	Emergency Room (n = 100)	Total (n = 400)
<12	1/2 (50)*	9/18 (50)	53/90 (59)	7/8 (88)	70/118 (59)
13–24	11/11 (100)	12/17 (71)	6/7 (86)	3/9 (33)	32/44 (73)
≤24	12/13 (92)	21/35 (60)	59/97 (61)	10/17 (59)	102/162 (63)
25–36	13/16 (82)	5/17 (29)	0/0	4/18 (22)	22/51 (43)
37–48	33/35 (94)	7/23 (30)	2/2 (100)	14/37 (38)	56/97 (58)
49–60	13/36 (36)	5/25 (20)	1/1	6/28 (21)	25/90 (28)
Total colonized	71†	38†	62	34†	205/400 (51)

*In parentheses: % colonized of all patients of same age.
†$P < 0.001$.

TABLE 2. Resistance Patterns to Various Antibiotics According to *S. pneumoniae* Serotypes

Serotype	No. Isolates	PEN	TMP/SMX	ERY	CLIN	TETR	CHL	CRO
6A	16	15/16 (94) 0/16*	13/16 (81)	15/16 (94)	15/16 (94)	15/16 (94)	0/16	0/16
6B	31	29/31 (94) 18/31 (58)*	28/31 (90)	27/31 (87)	28/31 (90)	24/31 (77)	0/31	0/31
11A	6	3/6 (50) 0/6*	2/6 (33)	0/6	0/6	0/6	0/6	0/6
14	20	20/20 (100) 9/20 (45)*	20/20 (100)	6/20 (30)	6/20 (30)	5/20 (25)	0/20	0/20
19A	11	11/11 (100) 0/11*	11/11 (100)	6/11 (55)	6/11 (55)	6/11 (55)	0/11	0/11
19F	26	24/26 (92) 16/26 (62)*	24/26 (92)	23/29 (79)	11/26 (42)	23/26 (88)	0/26	0/26
23F	52	48/52 (92) 35/52 (67)*	46/52 (88)	41/52 (79)	41/52 (79)	12/52 (23)	0/52	33/52 (63)

In parentheses: % of all isolates.
*High penicillin resistance (MIC [mtequ] 2.0 μg/mL).
PEN indicates penicillin; TMP/SMX, trimetoprim/sulfamethoxazole; ERY, erythromycin; CLIN, clindamycin; TETR, tetracycline; CHL, chloramphenicol; CR0, ceftriaxone.

Resistance to ≥3 antibiotic classes (MDR) was found in 138/205 (67%) isolates, and resistance for ≥5 antibiotic classes was found in 88/205 (43%) isolates. PCV7 and PCV10 covered 110/138 (80%) of MDR isolates. When the closely related serotype 6A was added, 123/138 (89%) of the MDR isolates were covered by PCV7 and PCV10. Ninety-three percent (129/138) of MDR isolates were covered by PCV13.

High percentages of nonsusceptibility to penicillin were recorded among serotypes 6A, 6B, 14, 19A, 19F, and 23F (94%, 94%, 100%, 100%, 92%, and 92%, respectively) (Table 2). High resistance to penicillin was recorded in serotypes 6B, 14, 19F, and 23F (58%, 45%, 62%, and 67% of all isolates, respectively). None of the 6A and 19A isolates was highly-resistant to penicillin. High resistance to ceftriaxone was found only among serotype 23F isolates (33/52, 63%).

Of the 41 nonvaccine serotype isolated, 23 (56%), 15 (37%), and 3 (7%) were susceptible, intermediate, and highly resistant to penicillin, respectively.

Of the 52 isolates of serotype 23F-*S. pneumoniae*, 4 (8%) were susceptible to penicillin. Of the 48 isolates nonsusceptible to penicillin, 35 (73%) had penicillin MIC ≥2 μg/mL. Thirty-three (63%) were nonsusceptible to ceftriaxone (MIC ≥ 4 μg/mL). All 52 isolates were susceptible to the 4 quinolones tested. Of the 23F-*S. pneumoniae* isolates, 49 (94%) were nonsusceptible to ≥3 antibiotic classes and 28/52 (54%) to ≥5 antibiotic classes.

DISCUSSION

The findings reported in this study on the NP colonization with *S. pneumoniae* in healthy children attending DCC, immunizations clinics or admitted for elective surgical procedures and sick children examined at the ER are not unexpected, taking into consideration the previous information available from 2 other regions of Romania.[1–3] The colonization rates with *S. pneumoniae* were age and sampling center-related, with the highest rates recorded in infants and children <2 years old and those attending DCCs. The distribution of the colonizing serotypes established the PCV7 serotypes 23F, 6B, 19F, and 14, and the vaccine related serotypes 6A and 19A as the leading serotypes recovered in the NP of the children enrolled in this area in Central Romania. The present analysis reveals lower PCV7 coverage of NP pneumococci isolated in Central Romania compared with that reported in pneumococcal diseases in United States, Canada, and Australia (80%–90%) or Europe (70%–75%).[5–7] On the other hand, our findings were similar to those reported on PCV7 coverage (42%–70%) of NP isolates in healthy children from many regions of the world.[5,6] The relatively low PCV7 coverage in the patients enrolled in this study could be explained, at least in part, by the older age of the patients.[5,6] The fact that PCV10 coverage was identical to that of PCV7 should not be surprising, since the 3 major invasive disease-causing serotypes (1, 5, and 7F) added in the PCV10 are, in fact, rarely detected in NP carriage studies.[5,6,8]

This study revealed extremely high rates of resistance of *S. pneumoniae* NP isolates, similar to those reported in other regions of Romania. Recently, Leibovitz et al[9] reported on the resistance patterns of *S. pneumoniae* isolated from patients with invasive diseases, AOM, and conjunctivitis, hospitalized in Iasi, Northeastern Romania; 74%, 86%, and 63% of invasive diseases, AOM, and conjunctivitis isolates, respectively, were nonsusceptible to penicillin.

In this study, 83% and 18% of all isolates were nonsusceptible to penicillin and ceftriaxone, respectively; furthermore, 40.5% and 16%, respectively, of all isolates were highly-resistant to penicillin and ceftriaxone. The nonsusceptibility rates to erythromycin, TMP/SMX, tetracyclin and clindamycin were also very high (>50% of all isolates for each antibiotic). Multidrug resistance was highly prevalent (67% for ≥3 antibiotic classes and 43% for ≥5 antibiotic classes). The majority of serotypes 6A, 6B, 14, 19A, 19F, and 23F were nonsusceptible to penicillin; high resistance to ceftriaxone was found only among serotype 23F isolates. The encouraging finding in this

study was that none of the vaccine-related serotypes 6A and 19A had MIC ≥2 μg/mL and that the great majority (80%–93%) of the MDR isolates belong to serotypes included in the pneumococcal conjugate vaccines.

Of the 52 isolates of 23F-*S. pneumoniae*, the majority had unusually high MIC values to penicillin and ceftriaxone. In a recent study characterizing 9 serotype 23-*S. pneumoniae* isolates from Bucharest, these isolates had very high penicillin, cefotaxime, cefuroxime, cefpodoxime, cefditoren, erythromycin, and gentamicin MICs.[10] All isolates were nonsusceptible to imipenem but susceptible to levofloxacin and vancomycin. MLST-predicted serotype was 23F in all but one strain (19F) but 3 strains were 19A by Quellung reaction. These Romanian strains presented a new cluster in the 595–600 region of PBP2X conferring 98% homology with *Streptococcus mitis* PBP2X. Although genotypic studies were not performed, the data emerging from our study raise the possibility of emergence and spread to different areas of Romania of similar pneumococcal strains with a breakthrough increase in the magnitude of β-lactam resistance.

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EFFECTIVENESS OF MEASLES VACCINATION FOR CONTROL OF EXPOSED CHILDREN

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Abstract: The effectiveness of measles vaccine for postexposure prophylaxis at educational centers was investigated. A total of 166 children who shared the classroom with 10 confirmed cases during the infectious period of cases were studied. Of total susceptible exposed children, 72% (54/75) were vaccinated and 25 contracted measles. Vaccine effectiveness in children vaccinated within 72 hours of exposure was 90.5% (95% confidence interval, 34%–99%).

Key Words: outbreak, measles, vaccine effectiveness, postexposure prophylaxis

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Measles is a highly infectious disease that can cause widespread outbreaks. The most effective preventive measure is vaccination with 2 doses of the trivalent measles, mumps, and rubella (MMR) vaccine. Vaccine uptake of at least 95% with 2 doses of MMR is considered to be necessary to achieve elimination.[1]

Although the objective of disease elimination in the European Region by 2010 was established, 120 outbreaks were reported in 2005 to 2008, 17 of which had more than 250 cases, and there were 25 deaths.[2]

If given within 72 hours of exposure to measles, immunization is the intervention of choice for the control of measles outbreaks in schools and child care centers.[1,3] However, the studies on which this recommendation is based are old and difficult to replicate.[4,5] More recent studies suggest that administering the MMR vaccine within 72 hours does not avoid the appearance of new cases.[6,7]

In Catalonia, a region in the northeast of Spain, a measles outbreak occurred between August 2006 and June 2007 causing 381 cases, mainly in infants aged <15 months.[8] The vaccination schedule at that time fixed the age of administration of 2 doses of vaccine at 15 months and 4 years. The objective of this study was to determine the vaccine effectiveness (VE) of the MMR vaccine as postexposure prophylaxis (PEP) in school children, taking into account different time periods from exposure to vaccination.

MATERIALS AND METHODS

We carried out a retrospective cohort study. The study population comprised children attending child-care centers and schools of the Barcelona-South Health Region (population 2,853,658 inhabitants) which a confirmed case of measles had attended during the infectious period.

A confirmed case of measles was a laboratory-confirmed case (positive serology for measles immunoglobulin M antibody by enzyme-linked immunosorbent assay testing or positive polymerase chain reaction for measles virus in urine sample) or a case that met the World Health Organization clinical case definition and was epidemiologically linked to a laboratory-confirmed case.

An index case was the first case of measles in the classroom; a contact was a child who had shared the same classroom as the index case for at least 1 day during the infectious period of the index case (4 days before rash onset to 4 days after); a secondary case was a contact with rash onset 7 to 18 days after rash onset in the index case. Candidates for the intervention were susceptible contacts (who had not received either measles-containing vaccine or had not suffered measles); intervention time was the period between rash onset of the index case and the day of vaccination of the susceptible contact.

Cases were investigated by public health staff. Susceptible contacts were identified and PEP immunization was offered. Active surveillance of centers was performed to detect secondary cases.

A network of public health clinics that maintain written immunizations records provided the immunity status to the regional public health units (either measles vaccination status or history of measles disease).

We obtained the secondary attack rate (SAR). Taylor series 95% confidence intervals (CIs) were calculated around the relative risk, and then calculated the VE and its 95% CI using the SAR base on the methodology described by Orenstein et al.[9] A multivariate analysis that used unconditional logistic regression was conducted. Analyses were performed using SPSS/PCv 18 and Epidat.

RESULTS

The contacts of 10 unvaccinated index cases (8 aged 6–14 months, and 2 aged 15 months–4 years) were included. The median infectious period in the classroom was 2 days (range, 1–4 days).

Exposed Contacts. In total, 166 children shared a classroom with the index cases, with a median age of 16.5 months (range, 6–47 months). The median class size was 14.5 children (range, 9–39). In all, 90 (54%) children had received 1 dose of MMR and 1 (1%) received 2 doses; 75 (45%) had received no dose nor had suffered measles.

Of the 75 candidates for the intervention, 25 contracted measles, of which 12 had received the vaccine as PEP. The median age of candidates was 12.2 months (range, 6–43 months).

Vaccine Effectiveness. Fifty-four susceptible contacts were vaccinated (72%). The median intervention time was 5 days (1–12 days).

The SAR among vaccinated children was 22% (12/54) compared with 62% (13/21) in unvaccinated children (relative risk, 0.4; 95% CI, 0.2–0.6). Among children who received the vaccine in ≤72 hours the SAR was 5.9% (1/17), with a VE of 90.5% (95% CI, 34%–99%) compared with unvaccinated children ($P < 0.001$). Administration of the vaccine was not effective in children who received the vaccine within 4 to 5 days of exposure (VE, 54%; 95% CI, 0%–81%), within 6 to 7 days (VE, 42%; 95% CI, 0%–73%) or later (Table 1). Neither age nor gender had any effect on the SAR (mentioned in Table, Supplemental Digital Content 1, http://links.lww.com/INF/A596). Multivariate analysis showed that the only association was with the administration of the measles vaccine within 72 hours (mentioned in Table, Supplemental Digital Content 2, http://links.lww.com/INF/A597).

DISCUSSION

The results of this study show that 1 dose of MMR vaccine reduces the risk of measles when administered in the 3 first days after rash onset in the index case, with an effectiveness of 90.5% (95% CI, 34%–99%), and are consistent with the results of the 2 previously-mentioned studies[4,5] and of a more recent one.[10]

Watson et al studied 4 susceptible members of the same family; 2 siblings received the vaccine a day after rash onset in the index case (3 days of exposure) and did not contract measles, whereas the 2 who were not vaccinated did contract the disease.[4] In the second report, Ruuskanen et al[5] evaluated the protective role of MMR administered during the 14 days after exposure among 5 school contacts. However, the time from exposure to vaccination was not detailed. Although the number of people included in these studies is low, in the most recent study by Sheppeard et al, in which 82 susceptible contacts were vaccinated within 3 days of exposure, no secondary cases were identified and the effectiveness of PEP with the MMR was 100%.[10]

Other studies found no protection as King et al[6] showing that the effectiveness conferred by MMR for PEP was negligible, but they could only administer the vaccine to 15 children, of which 9 were within 3 days after the onset of index case rash. This study can have an important memory bias because interviews were completed from 1 to 7 months after occurrence of illness. Moreover, authors suggest that these children had probably been exposed for 7 days before being vaccinated. Rice et al[7] in the United Kingdom administered MMR to 4 children who had been in close contact during the entire coryzal period of 1 case in a nursery, and all 4 children developed measles. The small number of subjects in both studies makes generalization of the results difficult.

In our study, the number of children exposed (75) and vaccinated (54) was higher than those of other studies[6,7] and showed that there were fewer cases in children vaccinated within 3 days than in unvaccinated children, and that in vaccinated children from day 4 onwards the point estimation of effectiveness was much lower, with a lower limit of nought.

For the implementation of the current recommendation of vaccination within 72 hours of exposure, 2 aspects have to be considered about the nature and timing of exposure. First of all, if the exposure has been continuous, it is not easy to determine when exactly it occurred. In field epidemiology, it is established the limit of 72 hours from the onset of rash in the index case. Second,

TABLE 1. Vaccination Status of Contacts, Secondary Attack Rate (SAR), and Vaccine Effectiveness (VE) According to Different Intervention Times in Educational Centers

Vaccination Status	No. Contacts	No. Secondary Cases	SAR (%)	RR (95% CI)	VE, % (95% CI)	*P*
Unvaccinated	21	13	61.9	1.0 (reference)	—	
Vaccinated	54	12	22.2	0.4 (0.2–0.6)	64.1 (34.5–80.3)	<0.001
≤3 d	17	1	5.9	0.1 (0.01–0.6)	90.5 (34.5–98.6)	<0.001
4–5 d	14	4	28.6	0.5 (0.2–1.1)	53.8 (0.0–81.1)	0.08
6–7 d	14	5	35.7	0.6 (0.3–1.3)	42.3 (0.0–73.5)	0.2
8–9 d	8	1	12.5	0.2 (0.03–1.3)	79.8 (0.0–96.9)	0.06
10–12 d	1	1	100.0	—	—	

RR indicates relative risk; CI, confidence interval.

compliance to the established period of time is difficult outside home. Even in an outbreak situation, when control measures are applied when a measles case is suspected (normally erythematous macular rash onset in a child with fever), and considering that children usually have fever 3 days before rash onset, and so are excluded from the educational center, the last exposure when the case is suspected is next to 72 hours. So, establishing vaccination of susceptible contacts in the centers cannot be achieved on the same day of the index case diagnosis. In this study, vaccination on the day after clinical suspicion was only achieved in 2 day-care centers (12 children) and only 17 of the 54 susceptible contacts were vaccinated within 72 hours. To carry out the intervention within the recommended time (72 hours), it is essential that the suspected case is reported urgently (less than 24 hours after the diagnosis), active surveillance is implemented and a close coordination between physicians and public health practitioners is held on.

Our results indicate that the recommendation of vaccinating susceptible exposed people in the first 72 hours should be continued. Vaccination of susceptible contacts acts in 2 ways. First, it provides direct immunity to the vaccinated person (avoiding secondary cases). Second, it increases herd immunity by increasing the proportion of immunized people, making transmission of the virus more difficult (avoiding tertiary and quaternary cases).

Although, unfortunately, herd immunity is no longer applicable once direct exposure occurs, the indirect benefit of measles vaccination in the community is clear, because disciplined use of measles vaccine can raise immunity levels above the threshold required to eliminate continued transmission in large populations.[11]

In conclusion, measles vaccine administered within 72 hours can be effective in preventing measles in susceptible contacts. However, given the difficulty of carrying out vaccination within this time, achieving and maintaining high routine vaccination coverages from 12 months onwards is the essential strategy to avoid measles outbreaks.

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THREE CHILDREN WITH PLASTIC BRONCHITIS ASSOCIATED WITH 2009 H1N1 INFLUENZA VIRUS INFECTION

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Abstract: We report the cases of 3 children with plastic bronchitis associated with 2009 H1N1 influenza virus infection. These 3 children shared common clinical and radiologic features: rapid and progressive respiratory distress with whole lung atelectasis on chest radiograph. In children with severe respiratory symptoms accompanied by H1N1 influenza, plastic bronchitis should be considered.

Key Words: 2009 H1N1 influenza virus infection, bronchial asthma, bronchial cast, bronchoscopy, plastic bronchitis

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The H1N1 influenza virus emerged in the spring of 2009 and spread globally, leading to an epidemic of influenza in Tokyo, last fall. Infection with this virus caused severe disease, requiring hospitalization, mainly in children and young adults.[1]

Plastic bronchitis is an uncommon condition characterized by bronchial casts typically containing fibrin exudates with varying amounts of inflammatory cells in the central airways, which induces acute respiratory failure.[2,3] It is most commonly associated with asthma, cystic fibrosis, and sickle-cell anemia, and can occur after surgical operation for congenital heart disease.[4,5]

This report describes 3 cases of plastic bronchitis in children with 2009 H1N1 influenza virus infection, who had severe dyspnea and whole lung atelectasis, requiring bronchoscopic removal of bronchial casts.

CASE REPORTS

Case 1. A previously healthy 2-year-old boy with a 2-day history of cough and a 1-day history of fever was transferred to the emergency department because of acute respiratory distress on September 20, 2009. He had been receiving oseltamivir therapy for influenza type A and was already intubated since the oxygen saturation was 70% in room air. In spite of no history of allergy or bronchial asthma, wheezing had sometimes occurred when he had had common colds. A chest radiograph showed complete opacification of the left hemithorax with a mediastinal shift to the left and increased pulmonary markings with hyperinflation of the right lung. Bronchoscopic examination showed redness of the mucous membranes of the trachea and the left bronchus, with voluminous serous discharge, but no obvious casts were detected. After repeated attempts at bronchial aspiration, a bronchial cast was removed on the day of admission, his condition improved, and the chest radiograph showed marked improvement. Histologically, the cast showed fibrinous exudates with a moderate amount of inflam-

matory cells consisting of 70% neutrophils, 20% macrophages, and 10% lymphocytes. He required mechanical ventilation for 11 days and received broad-spectrum antibiotics, systemic steroids, and a bronchodilator. His symptoms resolved, and he was discharged 18 days after admission. He continued to receive bronchodilator therapy.

Case 2. A 5-year-old boy was hospitalized with a 1-day history of cough, wheezing, and fever. The patient became increasingly dyspneic despite inhalation of salbutamol and intravenous infusion of steroids. He was transferred to our hospital on October 10, 2009, about 10 hours after admission to the previous hospital. Type A influenza was diagnosed, and he was treated with oseltamivir. He had been healthy with no history of bronchial asthma, but used to wheeze occasionally when he had had common colds. On physical examination, no breath sounds were audible over the left lung, and a chest radiograph showed complete opacification of the left lung, with a mediastinal shift to the left, hyperinflation, and increased pulmonary markings in the right lung (Fig. 1A). He was immediately intubated in the emergency department and underwent bronchoscopy, which demonstrated total obstruction of the main left bronchus by a large cast (Fig. 1C). The anatomy of the upper and lower lobes to the segment divisions was preserved. After removal of the cast, his respiratory condition improved, and the chest radiograph showed marked resolution of abnormalities (Fig. 1B). He required mechanical ventilation for 7 days and received intravenous fluids, antibiotics, bronchodilators, steroids, and oseltamivir. The histology of the cast was consistent with the typical features of plastic bronchitis: fibrinous exudates with abundant eosinophilic infiltration (Fig. 1D). The cellular content was 95% eosinophils, 2% neutrophils, 2% macrophages, and 1% lymphocytes. Charcot-Leyden crystals, which are often seen in sputum from patients with bronchial asthma, were absent. No bacteria were detected on culture. He was discharged without any complications 18 days after admission.

Case 3. A 6-year-old girl presented to a local clinic after 1 day of illness with fever, chest pain, and worsening dyspnea. A chest radiograph showed extensive pneumomediastinum and subcutaneous emphysema. She became increasingly dyspneic while waiting in the outpatient clinic and was transferred to our hospital on October 16, 2009. Physical examination showed diminished breath sounds over the left lung on auscultation, and the patient was tachypneic, dyspneic, and cyanotic. The rapid nasal swab diagnostic test showed type A influenza. She was intubated after admission, and casts were removed by subsequent aspiration. Bronchoscopy showed reddish, edematous mucous membranes of the respiratory tract. Histopathologic examination of the cast specimen revealed fibrinous exudates with a small amount of inflammatory infiltrates. The cellular components in the cast comprised approximately 40% neutrophils, 40% eosinophils, 15% macrophages, and 5% lymphocytes. On admission (3 hours after the previous radiographic examination of the chest), chest radiography showed atelectasis of the left lung, pneumomediastinum, and subcutaneous emphysema. The patient required mechanical ventilation for 6 days and received intravenous fluids, antibiotics, bronchodilators, steroids, and oseltamivir. She was discharged 11 days after admission. She had been well except for a history of bronchial asthma; the last attack was 1 month before admission. No bacteria were detected on culture of the aspirated specimen.

The 2009 H1N1 influenza infection was confirmed by polymerase-chain-reaction based testing in the all 3 patients.

DISCUSSION

To our knowledge, this is the first report of plastic bronchitis in children associated with 2009 H1N1 influenza infection in the

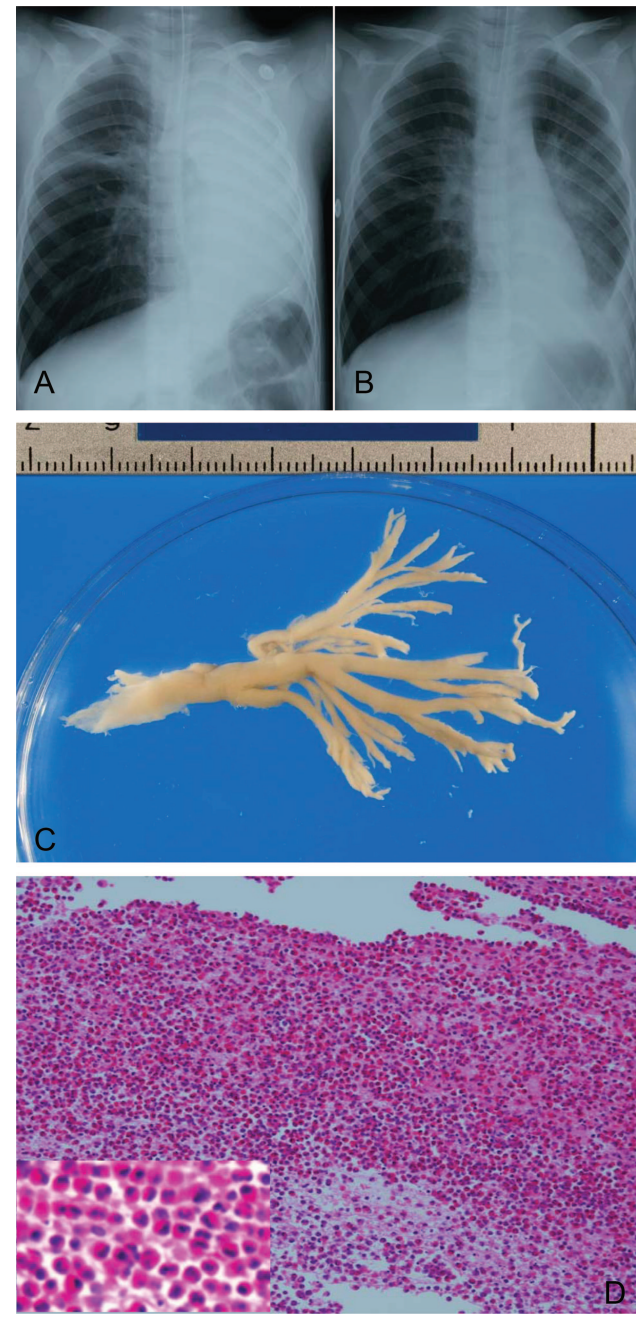

FIGURE 1. A, Chest radiograph in case 2. A chest radiograph obtained on admission, showing complete opacification of the left lung with a mediastinal shift to the left, hyperinflation, and increased pulmonary markings in the right lung. B, Chest radiograph in case 2. A chest radiograph obtained after bronchoscopy and removal of the cast, showing marked improvement. C, Gross findings in case 2. Cast measuring 6.5 × 4.3 cm, extracted from the left lung, demonstrating preserved anatomy of the upper and lower lobe to subsegment divisions. D, Histopathologic findings in case 2. Histopathology of the bronchial cast reveals a fibrinous exudate with eosinophilic infiltration. Inset shows a hyper-power view of eosinophils.

English language literature. Interestingly, these patients had plastic bronchitis, irrespective of the presence or absence of the known underlying diseases for plastic bronchitis; only 1 patient had bronchial asthma which is known to be an underlying cause for plastic bronchitis.[5] The other 2 patients had no obvious history of asthma or allergy, although they sometimes had wheezing when they had had common colds. We suggest that H1N1 influenza can be associated with plastic bronchitis, irrespective of presence or absence of underlying cardiopulmonary diseases. We also suggest that patients who have a history of wheezing episodes may be at higher risk for plastic bronchitis during the course of infection. Patients with wheezing may have similar pathologic conditions in the bronchus to those with asthma that underlie formation of bronchial casts. Those pathologic conditions in the central airway might include temperature, humidity, pressure, viscosity, or amount of fibrin or mucin exudates, degrees of inflammation, or immune response.

While 2009 H1N1 influenza infection induces acute respiratory distress syndrome in adults,[6] it might induce plastic bronchitis preferentially in children. The question arises as to why there have been no reports concerning plastic bronchitis in patients with 2009 H1N1 influenza infection. It is speculative that bronchoscopic examinations might not have been performed in most children who had severe respiratory distress, and repeated bronchoscopy may sometimes be required for detection of bronchial casts, as we experienced in case 1.

The time from symptom onset to admission was 1 to 2 days in our patients. The course is so rapid that anti-influenza virus agents may not be effective for the treatment of plastic bronchitis. Because patients with plastic bronchitis are at high risk for serious complications, admission to an intensive care unit is mandatory. One of the most effective treatments for plastic bronchitis is bronchoscopic removal of bronchial casts.

We conclude that in children with H1N1 influenza virus infection, who develop rapid and progressive respiratory distress with whole lung atelectasis, clinicians should be aware of the possibility of plastic bronchitis and consider bronchoscopic evaluation.

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PERSISTENCE OF HUMAN BOCAVIRUS DNA IN IMMUNOCOMPROMISED CHILDREN

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Abstract: Human bocavirus is frequently detected in immunocompetent as well as in immunocompromised children. However, the course of infection in immunocompromised children is still poorly investigated. In the present study, we describe 4 cases of repeat human bocavirus detection in the presence of severe immunodeficiency. In the view of homologous viral sequences identified in serial samples, possible persistence and reactivation in these patients are discussed.

Key Words: respiratory virus infection, human bocavirus, hematopoietic stem cell transplantation

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Viral acute respiratory infections represent a frequent complication among immunocompromised patients. Virus identification is relevant for preventive and therapeutic measures, including isolation and avoidance of unnecessary antibiotic therapies. The emergence of new respiratory viruses such as human bocavirus (HBoV)[1] in immunocompetent children raises questions regarding the significance of this newly described virus in the immunocompromised host.

In the present study, various respiratory samples were collected from immunocompromised pediatric patients treated at our institution in a 3-year period (2004–2007), whenever new respiratory symptoms occurred. The respiratory samples—together with nonrespiratory specimens (ie, plasma and feces) collected for other medical reasons around the time when respiratory symptoms began—were retrospectively screened by quantitative real-time polymerase chain reaction (PCR) for HBoV DNA.[2] Patients' charts were reviewed for relevant clinical data. Additionally, all respiratory specimens were screened for other respiratory viruses by multiplex PCR, using the ID-TAG Respiratory Viral Panel (TM Bioscience Corporation, Toronto, ON).[3] PCRs were performed prospectively on plasma samples for the detection of Epstein Barr virus (EBV) and cytomegalovirus (CMV). In total, 135 separate respiratory episodes occurring in 69 immunocompromised patients were included. In the present study, we describe 4 cases in which HBoV DNA was repeatedly detected during a prolonged period.

CASE REPORTS

Case 1. A 2-year-old girl with severe combined immunodeficiency syndrome was hospitalized in July 2004 with an EBV-associated B-cell lymphoma and active EBV infection. She presented with fever, lymphadenopathy, and mucosal infiltrates. Her general condition improved with rituximab and cidofovir treatment. Transplantation of allogenic hematopoietic stem cells (HSC) from an EBV-positive donor was planned. A reduced conditioning protocol (thiotepa, 5 mg/kg; fludarabine, 40 mg/m^2; and ATG) was adopted. With this regimen, the EBV DNA load declined from 153,000 to 7000 copies/mL and later was negative. However, the patient was severely granulocytopenic, and developed pulmonary aspergillosis. Pulmonary symptoms continued to deteriorate, despite aggressive antimicrobial and antifungal therapy. At this time, a nasopharyngeal aspirate (NPA), and 2 plasma samples taken 1 day before and 4 days thereafter, tested positive for HBoV (Fig. 1). Coinfection with rhinovirus was assessed by multiplex PCR.[3] A lung biopsy confirmed invasive aspergillosis with focal and intraalveolar bleeding and it also harbored HBoV DNA (Fig. 1). The patient

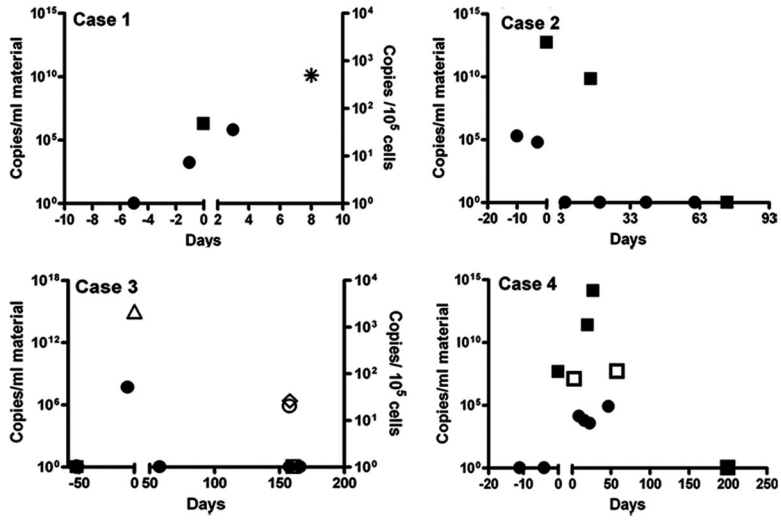

FIGURE 1. HBoV DNA load. HBoV quantification was performed in NPA (■), plasma (●), lung biopsy (*), BAL (Δ), sphenoid sinus biopsy (○), sphenoid sinus secretion (◇), and feces (□). Time point of first HBoV identification in respiratory samples is defined as day 0. Time points before or after day 0 are depicted as negative or positive figures, respectively.

eventually died of acute pulmonary decompensation and generalized capillary leakage.

Case 2. An 8-month-old EBV-positive boy with hemophagocytic lymphohistiocytosis was admitted to the bone marrow transplantation unit in July 2006. Treatment with cyclosporine A and steroids resulted in an increase of EBV DNA load from 518 to 9450 copies/mL in whole blood. The patient was treated with rituximab. At this time point, he also developed respiratory symptoms (tachypnea and supplemental oxygen requirement) with fever. Chest radiography showed lung infiltrates in the basal lower lobes. Two NPAs obtained at the beginning of the respiratory symptoms harbored high load of HBoV DNA (5×10^{12} and 7×10^9 copies/mL). Codetection of rhinovirus and human coronavirus NL63 RNA was assessed by multiplex PCR.[3] Viremia with low HBoV DNA load (1×10^2 and 2.3×10^3 copies /mL) was observed before (ie, day 11 and day 3) but not after HBoV detection in NPA (Fig. 1). The peak of HBoV DNA load in the respiratory tract was concomitant to EBV reactivation. The patient recovered, and subsequent serum samples and NPA were negative for HBoV DNA. Two weeks later, the patient underwent HSC transplantation and died of hepatic veno-occlusive disease.

Case 3. An 18-year-old boy with myelodysplastic syndrome and a history of HSC transplantation, completed 2 years previously, was hospitalized in March 2007 for acute respiratory symptoms. He presented with fever, productive cough, and tachypnea. Peribronchial infiltrates were evident on chest radiograph. The patient was under intensive immunosuppression to treat chronic GvHD of skin and mucosa. A bronchoalveolar lavage (BAL) and a serum sample taken at this time point were retrospectively tested for the presence of HBoV. The samples revealed extremely high DNA load in the BAL ($>10^{15}$ copies/mL) and relatively high load (4.7×10^7 copies /mL) in plasma. Additionally, the BAL sample was positive for PIV-3 in the multiplex PCR.[3] Microbiologic analysis of the BAL revealed the presence of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Aspergillus fumigatus*, as well as *Pneumocystis jirovecii* DNA, although no cysts were detected. Antifungal and antibacterial therapy was started and the patient's general condition improved. Later serum samples as well as an NPA and a stool sample were negative for HBoV (Fig. 1). The patient also had chronic sinusitis, and 5 months later underwent surgery for suspected aspergilloma. However, this diagnosis was not confirmed and microbiologic analysis of the sphenoid sinus biopsy and exudates was negative for the presence of fungi. Analyses of both materials for the presence of respiratory viruses revealed HBoV as the sole agent in the biopsy and HBoV and rhinovirus in the exudate. No NPA was available at the time of testing. Partial sequencing of the VP1 gene of HBoV from BAL, plasma, and sphenoid sinus samples was performed as described,[4] and revealed 100% identity. This finding is in accordance with persistence of the same HBoV strain over a 5-month period.

Case 4. A 4-year-old boy with dyskeratosis congenita developed pneumonia with perihilar infiltrates 17 days after HSCT. Repeat detection of high HBoV DNA load in NPA during a 2-month period with codetection of rhinovirus, which was accompanied by HBoV viremia and prolonged HBoV shedding (3 months) in the feces even after resolution of respiratory symptoms, has already been reported in detail.[5] As described previously, the patient showed a simultaneous CMV reactivation concomitant to the peak in HBoV load in NPS.[5] Partial sequencing of the VP1 gene of HBoV DNA obtained from the different samples was performed as described,[4] and revealed 100% identity, suggesting persistence of the same HBoV strain.

DISCUSSION

The present study shows that HBoV can be detected at moderate to high viral loads in samples from immunocompromised patients with underlying hematologic diseases or primary immunodeficiencies undergoing HSC transplantation. Previous studies have shown that prolonged HBoV shedding can be observed during immunosuppression, which possibly indicate persistence and/or reactivation in these patients.[5,6] A recent work also shows prolonged detection of HBoV in immunocompetent children with respiratory tract disease.[7]

In the 4 cases described in this study, we repeatedly detected HBoV DNA in the respiratory tract and/or in plasma, and in one case also in the gastrointestinal tract, for a period of up to 5 months. In all cases, HBoV replication in the respiratory tract was accompanied by viremia. However, the clinical relevance of prolonged detection of HBoV in respiratory samples and plasma is

unclear. Failure of the immune system in all 4 cases was characterized by severe granulocytopenia (below 500 granulocytes/μL) and/or impairment of both the T-cell and the B-cell compartment with opportunistic infection (aspergillosis) in case 1, EBV reactivation in case 2, aspergillosis and *P. jirovecii* infection in case 3, and CMV reactivation in case 4. This strongly indicates that severe immunodeficiency may lead to high levels of HBoV replication. Detection of HBoV in the lung biopsy of patient 1 should be interpreted with caution, because HBoV DNA was detected at the same time point in plasma, and we cannot exclude blood contamination of lung biopsy. Most intriguing was the detection of HBoV DNA in a sphenoid sinus biopsy 5 months after detection of more than 10^{15} and 10^{7} copies/mL in BAL and in plasma, respectively, in the absence of HBoV DNA in blood. HBoV may have played a role in the pathogenesis of chronic sinusitis in this case; alternatively, the mucosal tissue of nasal sinuses may represent a site of HBoV persistence after high-level replication in the respiratory tract. Similarly, recent findings have suggested that HBoV may establish persistent infections of mucosal lymphocytes and/or contribute to tonsillar hyperplasia in children.[8] Further investigations, with appropriate matched controls, are needed to understand the bystander or causative role of HBoV in chronic sinusitis in immunocompromised and immunocompetent patients.

In cases 1, 2, and 4, HBoV was detected during the late spring and summer months, whereas HBoV infections in immunocompetent patients predominantly have been described to occur during the winter season, and only rarely in spring or summer.[4] This observation, together with the fact that in these cases HBoV was detected after 3 to 5 weeks of strict isolation, further favors the hypothesis of reactivation of persistent HBoV infection during immunosuppression. Although it cannot be excluded, nosocomial infection appears to be less likely because of general prevention measures. The patient described in case 3 was 18 years old. Recent seroepidemiologic data suggest that antibodies to HBoV are present in more than 90% of children at the age of 6 years,[9] which makes primary infection in this patient unlikely. Moreover, sequence identity of HBoV DNA from samples taken 5 months apart was detected by partial sequencing of a region of the VP1 gene (819 nucleotides) known to display the greatest frequency of nucleotide polymorphisms.[4] This finding, further supported by identity of HBoV sequences in subsequent samples from a second patient (case 4), is compatible with persistent infection.

We found coinfections with one or more viruses in all 4 cases. A high frequency of codetection is a significant feature of HBoV and may argue against its causative role in respiratory infections. Moreover, Esposito et al[10] have recently shown that the clinical impact of HBoV in infected children may become significant when it is present together with other viruses. Potentially, HBoV may act as an exacerbating factor increasing the severity of infections caused by other pathogens.

Apart from the unsolved question of pathogenicity, our observations suggest the following 2 possibilities: (i) HBoV may be able to persist at low levels in the setting of an efficient immune system, thus making its detection difficult unless an immunocompromised status and/or coinfection with other viruses and subsequent increased replication occur; (ii) exposure to HBoV during immunosuppression can lead to persistent infection and prolonged viral shedding.

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ADVERSE NEUROLOGIC REACTIONS AFTER BOTH DOSES OF PANDEMIC H1N1 INFLUENZA VACCINE WITH OPTIC NEURITIS AND DEMYELINATION

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Abstract: When a neurologic condition develops after vaccination of a patient, the causal relationship is difficult to determine. We report an unusual case in which neurologic signs occurred in a previously healthy child after both doses of H1N1 2009 influenza vaccine, culminating in bilateral optic neuritis and disseminated encephalomyelitis. A causal association is more likely with repeated injury following influenza vaccination.

Key Words: optic neuritis, acute disseminated encephalomyelitis, post-vaccination, pandemic H1N1 influenza vaccine

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In response to the H1N1 pandemic of 2009, Canada deployed a domestically manufactured H1N1 vaccine adjuvanted with squalene and alpha tocopherol (Arepanrix, adjuvanted, inactivated, monovalent H1N1 vaccine, GSK Laval Quebec). Children, 6 to 35 months old, were recommended to receive 2 doses, at least 21 days apart. Surveillance activities in Canada and elsewhere detected rare instances of various demyelinating neurologic conditions in recently vaccinated persons. However, with vaccination programs occurring in the midst of an influenza pandemic, it was difficult to determine in individual cases whether the cause was infection, vaccination, or another etiology. In the absence of a diagnostic laboratory test for the trigger for demyelination, a useful but rare clinical clue to the cause is recurrence of injury upon re-exposure to the stimulus.[1,2] We report an unusual case in which neurologic

symptoms followed both doses of pandemic H1N1 influenza vaccine, culminating in bilateral optic neuritis and acute disseminated encephalomyelitis (ADEM).

CASE REPORT

A 2-year-old Filipino boy presented in late December 2009 with a history of unsteady gait starting 4 days after initial inactivated H1N1 influenza vaccination. His mother noted that he was unsteady while walking, leaning to his right side, and often falling. His mother took him to a local hospital where a computed tomography brain scan was done and read as normal. He was sent home and 4 days later his gait unsteadiness resolved. He remained well and was given his second H1N1 influenza vaccination 24 days after the first one. Six days later, his mother noticed that his left eye was turning in intermittently. This symptom resolved within 4 days. Eleven days after the second H1N1 influenza vaccination, his mother noticed that he was holding picture books much too close to see them.

No significant medical or family history was noted but 2 weeks before he received the first dose of H1N1 influenza vaccine, he had a febrile illness lasting for 3 days with cough and nausea. No diagnostic test was done; however, there was peak pandemic H1N1 influenza activity locally in British Columbia at that time.[3] Immunization dates were confirmed from a written record. The only vaccine given was Arepanrix.

On admission, the patient was afebrile with normal vital signs. He had evidence of myopia, as he needed to come close to objects to see them. Other than a mild, low amplitude, fast frequency action tremor present bilaterally, the remainder of the neurologic examination was normal. An ophthalmologist confirmed that his visual acuity was decreased. He seemed to prefer eccentric fixation, looking off to the side when viewing objects placed straight ahead of him. He had normal anterior segments but bilateral optic disc swelling, with venous engorgement and hemorrhages on the disc surface in the right eye.

A lumbar puncture was performed, and cerebrospinal fluid (CSF) analysis showed 2 mononuclear cells/μL with normal protein and glucose concentrations. His complete blood cell counts, serum chemistries, and liver enzymes were normal. Bacterial cultures of CSF were negative. Cranial magnetic resonance imaging performed on hospital day 2 demonstrated diffuse high intensity white matter lesions in the cerebellum and left basal ganglia, with increased signal in the optic nerves, consistent with ADEM. CSF polymerase chain reaction for enteroviruses, herpes simplex virus 1 and 2, and varicella zoster virus were negative. Further investigations including antinuclear antibodies, antineutrophil cytoplasmic antibodies, serum rheumatoid factor, human immunodeficiency virus serology, and IgM serology for Epstein-Barr virus and cytomegalovirus were negative. IgM serology for *Mycoplasma* was reactive, which was interpreted as infection in the past 6 months.

The patient's symptoms improved after a 5-day course of intravenous pulse methylprednisolone (30 mg/kg/d) treatment, whereupon his visual acuity improved and the optic nerve swelling resolved. He continued to improve without recurrence of symptoms. His neurologic examination was normal at 6-month follow-up.

DISCUSSION

The remarkable feature of this case was the occurrence of neurologic changes shortly after both doses of pandemic H1N1 vaccine, with spontaneous remission between episodes. The initial episode of gait unsteadiness eluded specific diagnosis, but was likely the first clinical manifestation of the cerebellar demyelination documented by his magnetic resonance imaging 3 weeks later. After the second vaccination, the presenting syndrome was dominated by bilateral optic neuritis. The unifying diagnosis proved to be acute disseminated encephalomyelitis (ADEM), which seemingly evolved in 2 stages, remitting after the first vaccination. This sequence of events is consistent with production of greater amounts of antibodies to myelin after each vaccination, perhaps aided by the presence of adjuvant in the vaccine that can induce potent nonspecific immune and inflammatory stimulation in animal models. The clinical significance of this is unknown.[4] Given the child's history of a febrile respiratory illness 3 weeks before initial vaccination, we cannot exclude the possibility that ADEM was initiated by pandemic influenza infection and exacerbated by the subsequent vaccinations. Another possibility that has been linked to ADEM and optic neuritis was *Mycoplasma* infection, for which serologic evidence existed for recent infection. However, the patient's neurologic symptoms occurred shortly after the first dose of vaccine and resolved and additional symptoms occurred shortly after the second dose of vaccine. This pattern strongly suggests that vaccine was a causative factor in producing these neurologic findings. Our case is consistent with the Brighton Collaboration case definition for ADEM with level 1 certainty.[5]

The most frequent side effects reported from pandemic H1N1 vaccine trials, related to the injection site.[6] Neurologic complications following influenza vaccination are uncommon, but various clinical syndromes have been reported such as encephalopathy, meningoencephalitis, Guillain-Barré syndrome, polyneuropathy, peripheral neuritis, and various combinations of these. The interval to onset varies from several hours to 6 weeks, perhaps starting late in the first week.[7,8]

ADEM is an inflammatory demyelinating disease of the central nervous system (CNS). ADEM may occur at any age but is more common in children and young adults. Approximately three-quarters of cases follow infection or immunization, as an encephalomyelitis. Postvaccination ADEM is associated with several vaccines such as rabies, diphtheria-tetanus-polio, smallpox, measles, mumps, rubella, Japanese B encephalitis, pertussis, hepatitis B, and influenza vaccine.[9] The incidence rates after most vaccines are as low as 0.1 to 0.2 per 100,000 vaccinated individuals.[10] However, there is no estimated incidence rate for ADEM after influenza vaccine and a causal relationship has not been established with certainty.[2]

The pathogenesis of ADEM is incompletely understood. The consensus is that an immune attack develops against CNS myelin.[11] It has been speculated that killed virus vaccines, such as trivalent influenza or swine influenza vaccines, may share similar antigens (molecular mimicry) with CNS proteins.[12] The viral antigens are believed to stimulate autoimmune reactions that produce CNS inflammation and demyelination.[13] A supportive study demonstrated induction of autoimmunity in healthy people after influenza vaccination, although this was of unknown clinical significance.[14] Other factors such as genetic predisposition may increase the odds of developing ADEM postvaccination.[9]

Optic neuritis may be a manifestation of ADEM. Optic neuritis after influenza vaccination is very rare and is a diagnosis of exclusion. Since 1971, 17 cases of optic neuropathy associated with influenza vaccination have been reported in the literature, including 2 associated with swine influenza vaccine and 1 recent case associated with trivalent, inactivated influenza vaccine.[9,12,15–19] Among these cases, our case is the youngest.

As in our case, most cases involve unilateral or bilateral optic neuropathy or ADEM, rather than simultaneous onset of both conditions. Most cases recover spontaneously or improve after

corticosteroid therapy, although permanent visual loss has been described in 2 cases with anterior ischemic optic neuropathy.[18]

A previous report described the occurrence of optic neuritis on 2 occasions after consecutive annual influenza vaccinations.[17] We recommended that our patient not receive annual influenza vaccination with a killed virus vaccine or a live-attenuated vaccine in the future, to avoid any recurrence risk. Early neurologic consultation and ophthalmologic evaluation should be considered in children presenting with these rare events.

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POWASSAN VIRUS INFECTION PRESENTING AS ACUTE DISSEMINATED ENCEPHALOMYELITIS IN TENNESSEE

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Abstract: Powassan virus is a rarely diagnosed cause of encephalitis, and is associated with significant neurologic sequelae. Although symptomatic infections with Powassan virus occur primarily in adults, we report a case of confirmed Powassan neuroinvasive disease in a child presenting to a Tennessee hospital, with symptoms and imaging studies suggestive of acute disseminated encephalomyelitis.

Key Words: flavivirus, Powassan, ADEM, encephalitis

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Powassan virus is an uncommon cause of arboviral encephalitis in North America, and has exclusively been identified in the Northern United States and Southeastern Canada. A 9-year-old female presented to the Emergency Department (ED) of Vanderbilt Children's Hospital in July of 2008 with fever and headache. She had been healthy until 3 days earlier when she noted the sudden onset of headache, abdominal pain, and emesis. Physical examination in the ED revealed fever to 103°F and nuchal rigidity, but no rash, arthritis, or conjunctivitis. Initial laboratory studies showed a peripheral WBC 19,500/μL with a differential of 90% neutrophils, 7% lymphocytes, and 3% monocytes; hematocrit of 44%; and platelet count of 237,000/μL. She had normal urinalysis, electrolytes, liver functions studies, and C-reactive protein of 4.5 mg/L (normal = 0.0–10.0 mg/L). A cerebrospinal fluid (CSF) examination revealed 68 nucleated cells (65% polymorphonuclear cells, 25% lymphocytes, 15% monocytes), no red blood cells, protein of 52 mg/dL, and glucose of 63 mg/dL. She was administered doxycycline, vancomycin, and ceftriaxone and admitted to the hospital.

The patient lived in Westchester County, NY and had 2 dogs, horses, gerbils, fish, and turtles. The patient had received a varicella vaccine booster 10 days before admission. She had attended a summer camp in rural New York State 10 days before admission, but she reported no known arthropod bites. The week before symptom onset she vacationed on the coast of Long Island, NY where she had cut her left foot while hunting for mussels in the ocean. She denied sick contacts, including those with recent meningitis or encephalitis.

During the first 3 hospital days, her fever persisted and her mental status, which was initially intact, declined to a Glasgow coma score of 12. With this decline, acyclovir was empirically added. She also developed bilateral ankle clonus, rigidity in her upper extremities, and a waxing and waning of her level of alertness. A magnetic resonance imaging (MRI) was performed on hospital day 5 which revealed multiple scattered T2 hyperintense lesions predominately affecting the white matter, with additional left putamen and caudate nucleus involvement (Fig. 1). The MR study showed no restricted diffusion, and no abnormal enhancement. These findings were

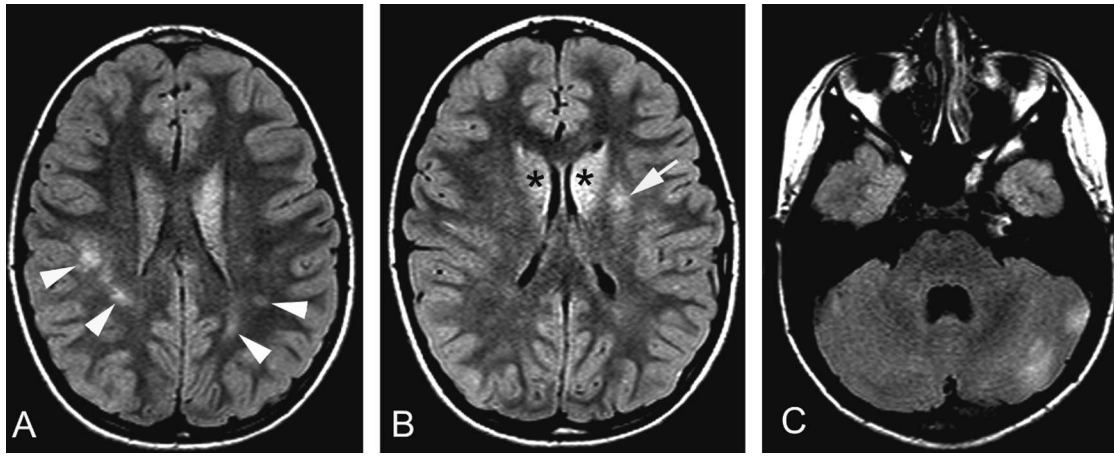

FIGURE 1. Axial T2-weighted FLAIR MR images in a patient with Powassan fever. Hyperintense signal is present in the (A) bilateral high cerebral white matter in a patchy distribution (white arrowheads), left caudate body, (B) bilateral caudate heads (black asterisks), left superior putamen (white arrow), and (C) left cerebellum white matter.

believed to be compatible with acute disseminated encephalomyelitis (ADEM), and she was treated with 500 mg methylprednisolone daily for 5 days. Over the next several days, she became afebrile and more alert, but her generalized weakness persisted. She was discharged after 9 days of total hospitalization with continued lower extremity weakness, imbalance, and persistent headaches.

Laboratory studies performed during her hospitalization included negative bacterial cultures of the blood and CSF; negative polymerase chain reaction studies on the CSF for herpes simplex virus, enteroviruses, adenovirus, Epstein-Barr virus, human herpes virus 6, varicella zoster virus, and La Crosse encephalitis virus. Acute serology was negative for Epstein-Barr virus, *Ehrlichia chaffeensis*, *Rickettsia rickettsii*, California encephalitis serogroup viruses, Eastern equine encephalitis virus, Western equine encephalitis virus, and West Nile virus. Acute serum tested positive for St. Louis encephalitis virus (SLE) with enzyme immunoassay (EIA) immunoglobulin M (IgM) elevated at 3.49 (>3.0 is positive). Additional testing performed by the Centers for Disease Control and Prevention, Atlanta, GA, revealed a Powassan virus-negative EIA IgG, but both a positive Powassan virus EIA IgM and plaque-neutralization test. Residual CSF was not available for testing at this time. Testing of convalescent serum identified Powassan virus IgG antibody by 2 methods. In microimmunofluorescence test, the median fluorescent intensity (MFI) bead cutoff was set at 1808 with acute sera MFI of 2641 and convalescent sera MFI of 16,330. Additionally, serum plaque-reduction neutralization tests were 10 on acute sera and 160 on convalescent sera. Both methods demonstrated a greater than 4-fold rise in activity thus confirming the diagnosis as acute Powassan virus infection.

At 3-month follow-up examination by a local neurologist in New York, she showed persistent subtle imbalance, but was overall greatly improved. On return to school, her grades were lower than when compared with the previous year. She had problems in mathematics, reading comprehension, and expressive reading. A follow-up MRI done 6 months after hospitalization showed almost complete resolution of the demyelination. At 15-month follow-up, her grades had returned to her previous standards although she still had subjective deficits in math and reading. She had returned to all of her normal activities including a martial arts class.

DISCUSSION

This is the first reported case of Powassan virus infection with a coincident, and potentially incorrect, diagnosis of ADEM. Radiographic findings with Powassan encephalitis are infrequently described. Previous reports of MRI findings consistent with acute ischemia or demyelination localizing to the parietal or temporal lobe have been reported, however, basal ganglia changes have not been previously described.[1] Powassan virus is a member of the Flaviviridae family of RNA viruses and is predominantly spread by ticks.[2] Other flaviviral encephalitides, including West Nile virus, Japanese encephalitis virus, tick-borne encephalitis virus, and SLE virus variably involve the basal ganglia and thalamus on MRI examination. For the majority of these flaviviruses, mosquitoes serve as the vector with an exception of tick-borne encephalitis virus, a flavivirus endemic to most of Europe and spread by the *Ixodes ricinus*. This infection is associated with lesions in the thalamus, putatmen, pallidum, and caudate nucleus on T2 MRI images.[3]

This patient's course was consistent with a classic presentation of Powassan encephalitis. The typical clinical presentation of Powassan central nervous system disease includes a 1 to 3 day prodrome of nonspecific symptoms such as sore throat, malaise, headache, and nausea, progressing to neurologic involvement, ranging from confusion to frank coma. Previous Powassan infections have been reported from May through December with the peak incidence reported from June through September.[2] In experimental conditions, unlike rickettsial illnesses, transmission of Powassan virus can occur within 30 minutes of tick attachment. Powassan virus has been isolated from a variety of ticks, including *Ixodes cookie*, *Ix. scapularis, Ix. marxi, Ix. spinipalpus,* and *Dermacentor andersonii*. Reported cases primarily occur in Southeast Ontario, New York, and Southern New England. A molecularly similar virus, deer tick encephalitis virus, has been recently reported in both the New England area and the Western Great Lakes[4] and has been isolated from *Ixodes dammini*. The amino acid homology is greater than 90% between these 2 viruses and it is currently

unclear whether Powassan virus and deer tick virus can be serologically distinguished.[5] Due to the conservation of major portions of the envelope protein, there is significant serologic cross-reactivity among flaviviruses. This was true in the case above, where the initial evidence of infection was based on a positive serologic test to SLE virus. Species specific neutralization assays are required to confirm individual infections.[6]

Case fatality rates of 10% to 15% for Powassan neuroinvasive infection have been reported. In a recent review, 11 of 20 infected patients had long-term neurologic sequelae, including headache, hemiplegia, and memory problems. Significant numbers of subclinical cases occur because up to 5% of individuals in endemic areas are seropositive for Powassan virus.[7]

The initial Powassan strain was isolated from a 5-year-old Canadian boy with fatal encephalitis in 1958 and the majority of early cases were reported in children and adolescents. Recently, however, of the reported cases, greater than 90% have occurred in adults.[1,2] The incidence of Powassan neuroinvasive infection may be increasing. In 2007, there were 6 cases of Powassan virus infection from New York State reported to the CDC, whereas in the previous 6 years, there had only been 1 case. Other states with sporadic cases in the last 8 years include Minnesota, Wisconsin, Michigan, and Maine. In 1999–2001, there was a mini-outbreak in Maine and Vermont.[1,5,8] Between 1958 and 1998, only 27 human cases were reported from Canada and the Northeast United States.[7] Whether the recent cases represent a true rise in incidence or reflect a surveillance artifact after the introduction of West Nile virus into the United States is uncertain.

This case highlights the need to include Powassan infection in the differential diagnosis of patients presenting with encephalitis. In the present case, the positive antibody to a related flavivirus, SLE virus, coupled with the history of recent exposure in an endemic area led to confirmatory testing for this unusual pathogen.

This case is the first to associate Powassan virus infection with basal ganglia MRI changes. It is unclear whether these changes were truly immune mediated as would be found in ADEM, or represented neurotropism of the Powassan virus. However, due to the clinical course and description of other flaviviral infections affecting the basal ganglia, it is most likely solely an effect of the Powassan virus infection. This is a significant issue, because although there is no antiviral treatment available, corticosteroids are often beneficial for treatment of ADEM but may be counter-indicated in acute Powassan viral infection.

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