DETECTION OF HUMAN CORONAVIRUS-NL63 IN CHILDREN IN JAPAN

Akira Suzuki, MD, Michiko Okamoto, BM,* Akira Ohmi,* Oshi Watanabe, MD,* Shigeaki Miyabayashi, MD, PhD,† and Hidekazu Nishimura, MD, PhD**

Abstract: Human coronavirus NL63 recently found in the Netherlands has been detected in Japan with a reverse transcription-polymerase chain reaction technique in clinical specimens from pediatric patients with respiratory symptoms. Of 419 specimens that were negative for common respiratory viruses, 5 were positive for human coronavirus NL63, and these specimens were all collected in the first 3 months of 2003.

Key Words: human coronavirus-NL63, human metapneumovirus, respiratory illness, Japan

Accepted for publication January 27, 2005.

From the *Virus Research Center and the †Department of Pediatrics, Sendai Medical Center, Sendai, Japan

Reprints not available.

DOI: 10.1097/01.inf.0000168846.71517.ee

Coronaviruses are enveloped, positive-stranded RNA viruses of animals, including humans, that cause respiratory or intestinal illnesses and that have been categorized into 3 groups/serotypes.[1] Two coronaviruses have been known since the 1960s as human coronavirus (HCoV)-229E and HCoV-OC43, belonging to groups 1 and 2, respectively. A fourth group is now the subject of discussion since the identification of severe acute respiratory syndrome-associated coronavirus, based on its genetic features.[2]

Recently, van der Hoek et al[3] reported the isolation of a new HCoV, belonging to group 1 coronaviruses, from a clinical specimen of a patient with respiratory illness. By full-genome analyses, the novel virus was revealed to be relatively close to, yet distinct from HCoV-229E. They named it human coronavirus NL63 (HCoV-NL63). In addition, they detected the viral gene using an reverse transcription (RT)-polymerase chain reaction (PCR) technique from 7 clinical specimens obtained from patients with respiratory infection during the 2002–2003 winter season, in the Netherlands. Soon after this, Fouchier et al[4] reported isolation of a "previously undescribed" human coronavirus "NL" (HCoV-NL) from a stocked clinical specimen obtained from a patient with respiratory infection in 1988 and detected this virus in 4 clinical specimens from patients with respiratory infection stocked in the 2000–2001 winter season, also in the Netherlands. The full genomes of HCoV-NL63 and HCoV-NL had 99% identity, indicating that these are essentially the same virus, although each was found through independent investigation, and that it had been existing since at least 1988 in that country. There is little epidemiologic and clinical information on this new HCoV, especially for areas other than the Netherlands. We succeeded in detecting this novel HCoV in children with respiratory infection and revealed the existence of this virus in Japan as well as collecting clinical information concerning the patients.

METHODS

This is a retrospective study with the use of throat and nasal swab specimens collected for virus isolation between January 2002 and December 2003, from pediatric patients who visited the outpatient clinic of the Sendai Medical Center, Sendai, Japan or were hospitalized in this hospital with respiratory infection.

Routine virus isolation was conducted by inoculation of each specimen onto a set of the following 5 kinds of cell cultures in 96-well microplates, a system intended to isolate common respiratory infection-related viruses[5]: Madin-Darby canine kidney; Vero; Hep-2; human embryo fibroblasts; and HMV-II cells. Specimens negative for virus isolation were kept at −80°C until this study was commenced.

The previously virus-negative specimens were subjected to RT-PCR to detect human metapneumovirus (hMPV) and HCoV-NL63 as follows. Total RNA was extracted from each specimen with the RNeasy kit (Qiagen, Hilden, Germany). The complementary DNA was obtained by reverse transcription (RT) reaction using random hexamers and Moloney mammary leukemia virus-RT (Invitrogen, Carlsbad, CA) as recommended by the manufacturer. The PCR was performed with the ExTaq kit (TaKaRa Bio, Otsu, Japan) and forward and reverse specific primers. For HCoV-NL63, we followed the method intended to detect the *1b* gene, described as diagnostic PCR by van der Hoek et al,[3] using 100 ng of primer repSZ-1 (5′-gtgatgcatatgctaattg-3′) and 100 ng of primer repSZ-3 (5′-ctcttgcaggtataatccta-3′). Our PCR consisted of the following steps: 95°C for 2 minutes; then 35 cycles of 94°C for 1 minute, 48°C for 1 minute and 72°C for 1 minute; then 72°C for 7 minutes. We did not attempt nested PCR for the detection of the *1b* gene. For hMPV, we performed nested PCR using specific primers to amplify a part of the *N* gene as described elsewhere.[6]

RESULTS

During the 2 years from January 2002 to December 2003, 1233 clinical specimens (male-female ratio, 656:577; outpatient-inpatient ratio, 840:393) were tested and 426 (34.5%) specimens were virus isolation- positive: 208 for influenza A virus (17 cases of H1N1 and 191 cases of H3N2), 1 for influenza B virus, 2 for influenza C virus, 53 for adenoviruses, 39 for respiratory syncytial virus, 20 for parainfluenza viruses, 7 for cytomegalovirus, 5 for rhinovirus, 3 for herpes simplex virus, 5 for enteroviruses, 2 for measles virus and 1 for mumps virus.

Virus isolation-negative specimens from our system were subjected to RT-PCR to detect hMPV and HCoV-NL63. One-half of the virus-negative specimens from each month were selected randomly by random number allocation (n = 419) and were subjected to the RT-PCR assay for hMPV and HCoV-NL63.

Of the 419 specimens tested, 5 were positive for HCoV-NL63. The positive results by PCR were confirmed by their nucleotide sequences, and the partial sequences of their *1b* gene were deposited to GenBank under accession numbers AY662694–AY662698, respectively. All were identical with each other over the 169-nucleotide sequence and were also identical with those detected in the Netherlands[3,4] (GenBank accession numbers AY567487 and AY518894) except for 1 synonymous difference. Forty-six specimens were positive for hMPV, but the 5 HCoV-NL63-positive specimens were negative for hMPV.

The HCoV-NL63-positive specimens were collected during a short period between January and March 2003, showing a similar tendency to the previous 2 works in which HCoV-NL63 were detected mostly from the specimens collected in the winter season.[3,4] However, we could not detect HCoV-NL63 in 2002, although a comparable number of specimens were tested for each month.

The HCoV-NL63-positive patients were ages 6 months–9 years (mean, 55 months), and the male-female ratio was 2:3. Two patients had possible predisposing factors: a 6-month-old girl born at 29 weeks of gestation birth at a weight of 1902 g; and an 8-year-old girl with hereditary spherocytosis who had had splenectomy. Clinical symptoms among them were: body temperature >38.5°C, 5 of 5; rhinorrhea, 4 of 5; cough, 4 of 5; and wheezing, 1 of 5. Four of them were outpatients diagnosed clinically with an upper respiratory

infection, and one 9-month-old boy was hospitalized with "asthmatic bronchiolitis." A chest radiograph of the latter patient obtained on admission showed bilateral parahilar infiltrates. Bacterial examination led to the isolation of *Moraxella catarrhalis* from his throat swab and a slightly elevated serum C-reactive protein value (6.5 mg/dL).

DISCUSSION

We found that HCoV-NL63 exists not only in the Netherlands but also in a community within Japan in pediatric patients with respiratory infection of unknown etiology.

The results of our partial nucleotide sequencing of the *1b* gene showed almost perfect conservation compared with those reported from the Netherlands,[3,4] indicating that, at least, this part of the *1b* gene might be a good target for detection of this virus by RT-PCR, as suggested by van der Hoek et al.[4]

Detection was successful only from specimens obtained between January and March 2003 in 18% of specimens tested. We cannot make an accurate assessment as to the prevalence of this virus at the community level, because we neither know about its prevalence in children without respiratory symptoms nor have access to samples collected from adults.

We were unable to detect the virus in specimens collected in 2002, including a comparable number of specimens in the first 3 months. This suggests that outbreaks of this virus infection do not necessarily occur every season, unlike those of influenza. As for HCoV-229E, it has been reported that outbreaks occurred at 2- to 4-year intervals, whereas those of HCoV-OC43 occurred every second year.[7]

Van der Hoek et al[3] reported that patients infected with HCoV-NL63 had upper or lower respiratory tract infections, and Fouchier et al[4] isolated the virus from a 5-month-old pneumonia patient. Four of 5 of our HCoV-NL63-positive patients had mild, nonspecific symptoms. Although we did not have definitive evidence, it seems probable that the symptoms of our patients were caused by this virus because the specimens from all these patients were negative for common viruses including hMPV.

We had an admitted case of asthmatic bronchiolitis, later found to be HCoV-NL63 positive which might be interesting in relation to a report suggesting a possible role of viral infection as a trigger for asthma exacerbation.[8] However, *M. catarrhalis* was also isolated from this patient.

HCoV-229E and OC-43 were reported to cause mild colds in human adult volunteers,[9] lower respiratory tract infections in the pediatric population,[10] and severe acute respiratory syndrome-associated coronavirus has caused fatal pneumonia in adults.[2] On the other hand, research of HCoV-NL63 infection has just started, and there is little clinical information and no solid pathologic or statistical data that could strictly prove the significance of this novel virus as a respiratory pathogen.

ACKNOWLEDGMENTS

We thank Wakiko Morikwa, Ayaka Ochiai, Hiroko Ito, Fumiko Chiba and Izumi Tanaka for their assistance.

REFERENCES

1. Lai MMC, Holmes KV. Coronaviridae. In: Knipe DM, Howley PM, ed. *Field's Virology*. Philadelphia, PA: Lippincott-Raven Publishers; 2001: 1163–1203.
2. Gorbalenya AE, Snijder EJ, Spaan JM. Severe acute respiratory syndrome coronavirus phylogeny: toward consensus. *J Virol*. 2004;78: 7863–7866.
3. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. *Nat Med*. 2004;10:368–373.
4. Fouchier RA, Hartwig NG, Bestebroer TM, et al. A previously undescribed coronavirus associated with respiratory disease in humans. *Proc Natl Acad Sci U SA*. 2004;101:6212–6216.
5. Numazaki Y, Oshima T, Ohmi A, et al. A microplate method for isolation of viruses from infants and children with acute respiratory infections. *Microbiol Immunol*. 1987;31:1085–1095.
6. Suzuki A, Watanabe O, Nishimura H. Detection of human metapneumovirus from wheezing children in Japan. *J Jpn Assoc Infect Dis*. 2003;77:467–468.
7. Monto AS, Lim SK. The Tecumseh study of respiratory illness, VI: Frequency of and relationship between outbreaks of coronavirus infection. *J Infect Dis*. 1974;129:271–276.
8. Tuffaha A, Gern JE, Lemanske RF Jr. The role of respiratory viruses in acute and chronic asthma. *Clin Chest Med*. 2000;21:289–300.
9. Bradburne AF, Bynoe ML, Tyrrell DA. Effects of a "new" human respiratory virus in volunteers. *Br Med J*. 1967;3:767–769.
10. McIntosh K. Coronavirus infection in acute lower respiratory tract disease of infants. *J Infect Dis*. 1974;130:502–507.

ROTAVIRUS EPIDEMIOLOGY IN SAN LUIS POTOSÍ, MEXICO

Daniel E. Noyola, MD,† and Ismael F. Herrera, MD**

Abstract: The epidemiology of rotavirus infections was investigated in San Luis Potosí, Mexico during a 6-year period. In each of the study years, the epidemic period started in October or November; peak activity was detected between December and February, and the end of the epidemic occurred in March or April. Rotavirus infections show a consistent temporal pattern in our community.

Key Words: rotavirus, epidemiology, gastroenteritis, Mexico
Accepted for publication January 11, 2005.
From the *Microbiology Department, Facultad de Medicina, Universidad Autónoma de San Luis Potosí, and the †Pediatrics Department, Hospital Central "Dr. Ignacio Morones Prieto," San Luis Potosí, Mexico
Reprints not available.
DOI: 10.1097/01.inf.0000168841.84462.ad

It is well-established that rotavirus is the main etiology for acute watery diarrhea (up to 60% of cases) in industrialized countries, as well as in developing countries. In the latter, rotavirus is the main pathogen that can be identified in children younger than 2 years of age with severe gastroenteritis and those requiring hospitalization.[1–4] Worldwide it is estimated that in children younger than 5 years of age up to 111 million cases of rotavirus infections occur each year requiring home care only; 25 million cases require medical attention; 2 million children are hospitalized; and there are 352,000–592,000 related deaths.[5]

The incidence and epidemiologic patterns of rotavirus infection vary among different geographic regions. In temperate regions, the peak activity is recorded during the colder months of the year, fall and winter.[6–11] Sporadic cases occur during the summer months. In tropical areas, this seasonal pattern is not always present. In Mexico, rotavirus infections are reported during the fall (peak activity reported in October and November) with continued activity during the winter and even into spring.[12,13] LeBaron et al[10] reported the epidemic pattern of rotavirus infections in North America between 1984 and 1988. In that report, the peak activity of rotavirus infection in Mexico was in October and November. That study was from a single center in Mexico (located in Mexico City). Just as there are differences in seasonal patterns for rotavirus infections in different areas of the United States, it is expected that there may be geographic variations in Mexico. It is important to determine these seasonal variations throughout different areas, especially taking into account the proximate availability of rotavirus vaccines. We per-

formed a retrospective study to determine the seasonal patterns of rotavirus infection in the capital city of the state of San Luis Potosí, Mexico. San Luis Potosí is located in the center of Mexico and has a semiarid climate. The objectives of the study were to determine the epidemic curve of rotavirus infection in our city and to compare the pattern observed throughout a 6-year period.

METHODS

We included data from 5 clinical laboratories that perform rotavirus antigen detection. Participating laboratories included the laboratory at the Hospital Central "Dr. Ignacio Morones Prieto" (a public, reference, general hospital) and 4 private laboratories that we considered to be representative for the city. The Hospital Central "Dr. Ignacio Morones Prieto" serves economically disadvantaged populations and has inpatient and outpatient services. The private laboratories predominantly serve middle and upper class ambulatory patients.

Data Collection. Each of the participating laboratories was contacted, and information regarding the weekly number of stool samples processed for rotavirus detection and the number of positive samples was requested. The total number of tested and positive samples was determined by combining data from participating laboratories. Stool specimens processed from July 1998 through June 2004 were included in the study. Results were recorded for each epidemiologic week as defined by the Dirección General de Epidemiología, Secretaría de Salud, Mexico. Rotavirus detection assays used included latex agglutination tests (BioMérieux, Marcy l'Etoile, France; Rotalex, Orion Diagnostica, Espoo, Finland; and Meritec-Rotavirus, Meridian Bioscience, Cincinnati, OH) and enzyme immunoassay (ImmunoCard Stat! Rotavirus; Meridian Bioscience, Cincinnati, OH).

Definitions. The year corresponding to each rotavirus epidemic was defined as the time lapsed between June of the corresponding year and July of the following year. Onset for the epidemic period was defined as the first of 2 consecutive weeks in which the number of positive samples exceeded 10% of tested specimens, and there were at least 2 positive samples during each week. The peak of the season was defined as the 4 consecutive weeks when the greatest number of positive samples for the epidemic period were detected. The end of the rotavirus season was defined as the last of 2 consecutive weeks in which the number of positive samples exceeded 10% of tested specimens and there were at least 2 positive samples in each week. During the 2000 rotavirus season, the duration of rotavirus disease was very prolonged, presenting a biphasic distribution including 2 epidemic periods separated by 4 consecutive weeks in which the number of positive samples did not exceed 10%, and in none of them there was more than a single positive sample. For that year, we considered that there were 2 epidemic periods; the first was designated the main episode and was used for comparison with the other years; subsequently detected cases represented a secondary minor outbreak presenting during the summer.

RESULTS

During the 6-year study period, there were 3716 specimens tested at the participating laboratories with 1787 (48%) samples reported as positive for rotavirus. Participating laboratories provided data for all years included in the study, except 2 laboratories that did not provided information for 1 (1998) and 2 (1998 and 1999) seasons, respectively. The yearly number of positive samples ranged from 232 to 431 (average, 298 samples).

The epidemic curve for rotavirus activity in San Luis Potosí is shown in Figure 1. With the exception of the year 2000 epidemic that showed a biphasic pattern with a secondary minor outbreak during the summer, all years showed a single peak during the cold months of the year and sporadic cases during the spring and summer. The onset of the epidemic period was recorded consistently in October or November of all years. The epidemic ended in March or April. The peak activity was registered between December and February. Onset of peak rotavirus activity was detected from December to January (earliest peak onset, December 10). The end of peak rotavirus activity occurred in January or February (latest end of peak activity, February 14). The number of positive rotavirus samples during the epidemic period included 91.4–98.3% of all positive tests for a given year.

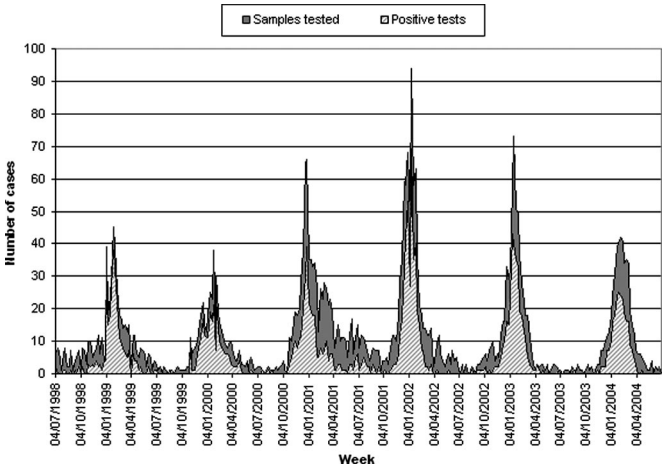

FIGURE 1. Rotavirus epidemiology in San Luis Potosí, Mexico, from July 1998 through June 2004.

DISCUSSION

We have studied the epidemiologic features of rotavirus infections in our city during a 6-year period. Our results show a rather consistent temporal presentation for rotavirus infections in our community. In each of the 6 consecutive years, the epidemic period started late in October or in November. Peak activity onset varied between December and January, but January was always included among the 4 peak weeks for rotavirus detection. End of the epidemic occurred in March or April. Our findings differ from those reported by LeBaron et al[10] in which peak activity in Mexico was reported to occur in October–November. Our results, which were consistent throughout a 6-year study period, show a presentation pattern closer to that observed in Northwest and Midcentral states of the United States.[10] The differences in rotavirus activity observed in our study and that reported in Mexico City could be explained by several factors, including differences in geographic locations, climate and period of time included in the study. These observations are useful as a baseline to assess the effect of rotavirus and rotavirus vaccines on gastrointestinal disease in our community.

ACKNOWLEDGMENTS

We thank María del Socorro García-Luna, PhD (Centro de Diagnóstico in Vitro), Marta Sánchez de la Barquera, MD (Laboratorio Glosan), Guillermo Castro Patton, QFB (Laboratorio Castro Patton), Jesús Rosillo de León, MD (Laboratorio de Tequis) and Laboratorio Clínico Hospital Central "Dr. Ignacio Morones Prieto" for providing information for this study.

REFERENCES

1. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ*. 2003;81:197–204.

2. Bingnan F, Unicomb L, Rahim Z, et al. Rotavirus-associated diarrhea in rural Bangladesh: two-year study of incidence and serotype distribution. *J Clin Microbiol*. 1991;29:1359–1363.
3. Black RE, Merson MH, Rahman AS, et al. A two-year study of bacterial, viral and parasitic agents associated with diarrhea in rural Bangladesh. *J Infect Dis*. 1980;142:660–664.
4. Kapikian AZ. Viral gastroenteritis. *JAMA*. 1993;269:627–630.
5. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis*. 2003;9:565–572.
6. Bartlett AV 3rd, Reves RR, Pickering LK. Rotavirus in infant-toddler day care centers: epidemiology relevant to disease control strategies. *J Pediatr*. 1988;113:435–441.
7. Kapikian AZ, Kim HW, Wyatt RG, et al. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. *N Engl J Med*. 1976;294:965–972.
8. Houly C, Grunert B, Werchau H, Mietens C, Brussow H, Hilpert H. Epidemiology of rotavirus gastroenteritis in infants from the area of Bochum as revealed by electrophoresis of genome RNA. *Eur J Pediatr*. 1984;143:128–132.
9. Konno T, Suzuki H, Katsushima N, et al. Influence of temperature and relative humidity on human rotavirus infection in Japan. *J Infect Dis*. 1983;147:125–128.
10. LeBaron CW, Lew J, Glass RI, Weber JM, Ruiz-Palacios GM. Annual rotavirus epidemic patterns in North America: results of a 5-year retrospective survey of 88 centers in Canada, Mexico and the United States. *JAMA*. 1990;264:983–988.
11. Matson DO, Estes MK, Burns JW, et al. Serotype variation of human group A rotaviruses in two regions of the United States. *J Infect Dis*. 1990;162:605–614.
12. Raul Velazquez F, Calva JJ, Lourdes Guerrero M, et al. Cohort study of rotavirus serotype patterns in symptomatic and asymptomatic infections in Mexican children. *Pediatr Infect Dis J*. 1993;12:54–61.
13. Villa S, Guiscafre H, Martinez H, Muñoz O, Gutierrez G. Seasonal diarrhoeal mortality among Mexican children. *Bull World Health Organ*. 1999;77:375–380.

OUTBREAK OF EXTENDED SPECTRUM β-LACTAMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* INFECTION IN A NEONATAL INTENSIVE CARE UNIT RELATED TO ONYCHOMYCOSIS IN A HEALTH CARE WORKER

Icaro Boszczowski, MD, Christiane Nicoletti, MD, MSC,* Dilma M. T. Puccini, RN,* Mônica Pinheiro, MD,* Robson E. Soares,† Inneke M. Van Der Heijden,† Silvia F. Costa, MD, PhD,† Antonio A. Barone, MD, PhD,† and Anna S. Levin, MD, PhD†*

Abstract: Four cases of infection by extended spectrum β-lactamase-producing *Klebsiella pneumoniae* occurred in the neonatal intensive care unit. Isolation, empiric therapy change and education produced no effect. Newborn weekly colonization rates were 0–18.7%. One health care worker with onychomycosis was positive for extended spectrum β-lactamase-producing *K. pneumoniae*. Isolates were identical by molecular typing. Outbreak was controlled when the health care worker was excluded from the neonatal intensive care unit.

Key Words: *Klebsiella pneumoniae,* onychomycosis, health care worker

Accepted for publication January 18, 2005.

From the *Hospital Geral de Itapecerica da Serra and the †Department of Infectious Diseases and Laboratory of Medical Investigation (LIM 54), University of São Paulo, São Paulo, Brazil

Address for reprints: Icaro Boszczowski, Rua Joao Moura, 975/192, São Paulo-SP 05412-002, Brazil. E-mail icarobski@uol.com.br.

DOI: 10.1097/01.inf.0000168844.55898.8f

During the past decades, more newborns weighing <1000 g have survived, and this larger population of premature infants has experienced an increase in infection in neonatal intensive care units (NICU).[1]

Premature neonates with an immature immunologic response and subjected to invasive devices such as catheters and mechanical ventilation are at increased risk of infection and colonization by nosocomial microorganisms.[2] Several outbreaks caused by extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* have been described.[3–6] ESBLs are generally plasmid-encoded β-lactamases present in Gram-negative bacteria that confer resistance to most β-lactams except for carbapenems and cephamycins. Risk factors for acquiring *K. pneumoniae* in the NICU are low birth weight, parenteral nutrition, use of blood products and central venous catheters.[7]

This study is a description of a nosocomial outbreak of *K. pneumoniae* associated with persistent hand carriage by a health care worker.

METHODS

The outbreak occurred in the NICU of a general community hospital in Itapecerica da Serra in the State of São Paulo, Brazil. The unit has 20 beds: 8 for intensive care; 10 for intermediate care; and 2 isolation rooms, distributed in 4 areas.

In July 1999, 4 cases of nosocomial infection by *K. pneumoniae* resistant to cephalosporins and aminoglycosides occurred; 3 infants had bacteremia and 1 had urinary tract infection.

The following measures were introduced by the infection control team: weekly surveillance cultures (anal, nasal and oropharyngeal) of all newly admitted patients to the unit; contact isolation of all patients colonized by ESBL-producing *K. pneumoniae*; the patients initially negative were cultured weekly until discharge from the unit or turning positive; the protocol for empiric treatment of late onset sepsis was changed from amikacin and oxacillin to imipenem; education of all the staff regarding adequate patient care techniques such as catheter and ventilator care, hand and patient hygiene; the colonization rate was calculated on a weekly basis dividing the number of new positive cases of colonization by the number of newborns cultured.

After this intervention, new cases continued to appear. In October 1999, hand cultures of all health care workers were carried out for the purpose of hand hygiene practices.

MICROBIOLOGY

Cultures of the hands of health care workers were obtained via swabs with Stuart medium. They were plated on selective MacConkey medium. Initially identification and antimicrobial susceptibility testing were conducted with an automated system (Vitek; BioMérieux), and ESBL production was evaluated with the disk diffusion and E-test methods as described by the Clinical and Laboratory Standards Institute.[8–10] Later identification was confirmed using biochemical tests and antimicrobial susceptibility was evaluated for imipenem, ciprofloxacin, ceftriaxone, ceftazidime, cefoxitin, cefotaxime and cefoperazone by broth microdilution.[11]

Surveillance cultures of the infants were done by anal, nasal and oropharyngeal swabs using sterile saline. Isolation, identification and susceptibility testing were performed as described above.

Molecular typing of isolates that colonized 11 patients and 1 from a positive health care worker was performed by pulsed field gel electrophoresis with restriction enzymes *Xba*I and *Spe*I as described elsewhere[12] *K. pneumoniae* previously known was used as a control.

RESULTS

Twenty-five cultures were obtained from health care workers: 8 from doctors; 4 from nurses; and 13 from nurse assistants. The hands of 1 nurse assistant were positive for ESBL-producing *K. pneumoniae*. She presented signs of extensive onychomycosis on the right thumb and was temporarily removed from patient care. After that, there were no new surveillance cultures positive in the NICU until the health care worker returned (5 weeks later). After her return to the unit, new cases were identified, and she was excluded from the NICU for treatment of her condition. Two months later, she continued to be positive for *K. pneumoniae*. For 6 weeks after that, there were no more new cases, and the outbreak was considered ended (Fig. 1). Empiric treatment of late onset sepsis returned to amikacin and oxacillin.

The only cases of disease occurred in July 1999 when the outbreak was identified with 4 cases of invasive disease: 3 patients with bacteremia; and 1 with urinary tract infection. During the period of active surveillance, there were only cases of colonization. Weekly colonization rates varied from 0 to 18.7%.

Molecular typing demonstrated that the isolates from patients and the health care worker were identical (Fig. 2).

All isolates were susceptible to imipenem and ciprofloxacin, and 57% were susceptible to ceftazidime and 42% to cefoxitin.

DISCUSSION

Persistent carriage on the hands of health care workers as the source of outbreaks caused by Gram-negative rods is relatively uncommon. Although in 1980 there was a description of an outbreak by *Citrobacter diversus* in which the hands of a nurse with dermatitis were implicated,[13] it is more common that the sources of Gram-negative outbreaks are environment-related.[14–18] *Staphylococcus aureus* is the microorganism most implicated in outbreaks traced back to a health care worker (HCW) as the source. The colonization of the anterior nares acts as a reservoir of *S. aureus* and is a risk factor for surgical site and burn wound infections.[19–22] In such outbreaks, respiratory tract infections in the colonized HCW associated or not with hand carriage is a risk factor for infections in patients.

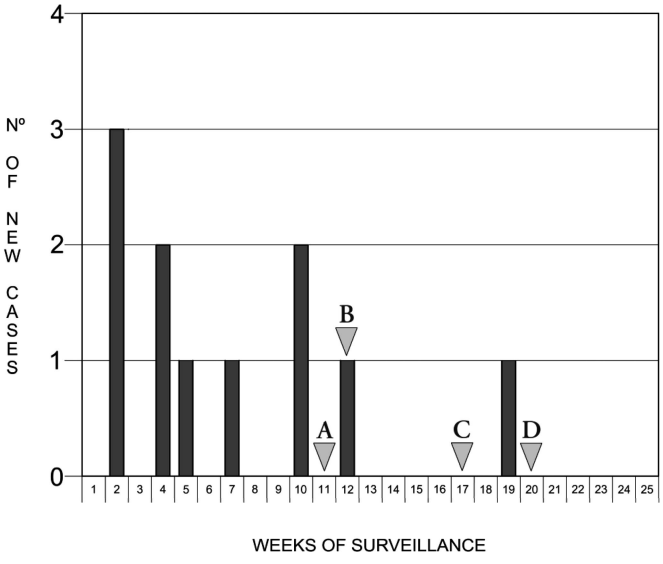

FIGURE 1. Number of weekly new cases of colonization by ESBL-producing *K. pneumoniae* identified by active surveillance of patients in the NICU.

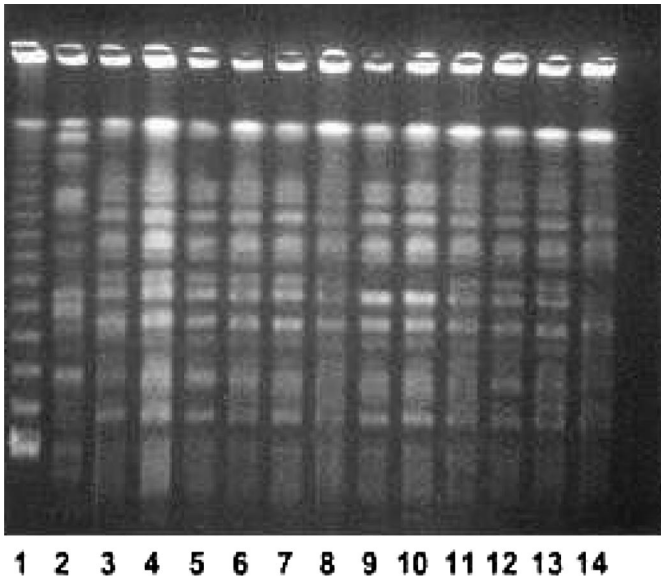

FIGURE 2. Results of molecular typing of ESBL-producing *K. pneumoniae* involved in a an outbreak in a neonatal intensive care unit. Lane 1 indicates λ ladder molecular weight marker; lane 2, nonrelated control *K. pneumoniae*; lane 3, isolate from health care worker; lanes 4–14, isolates from colonized patients.

Among outbreaks caused by Gram-negative rods with an identified environmental source, nonfermenters predominate. The source include thermometers;[14] taps;[15] and hydrocoloid dressings.[16] Other examples of environmental sources are *Legionella* spp. in water,[23] *Salmonella* spp. in nutrition,[24] *K. pneumoniae* in human milk suction devices,[25] thermometers[26] and ultrasonography gel.[17]

The use of artificial fingernails has been associated with persistent colonization of HCW in outbreaks of *Pseudomonas aeruginosa*[27,28] and *K. pneumoniae.*[29] The presence of dermatits as a factor leading to persistent colonization of HCWs has been described in outbreaks of *S. aureus*,[21] *C. diversus*,[13] *Acinetobacter calcoaceticus,*[30] and *P. aeruginosa.*[31] The occurrence of dermatitis on hands can lead to lower adherence to practices of hand hygiene.[32] There have been descriptions of pseudooutbreaks caused by *Streptococcus pyogenes* and *S. aureus* attributed to impetigo and cellulitis in the hands of professionals working in the microbiology laboratory.[33] Onychomycosis has been implicated as a factor associated with persistent colonization with *P. aeruginosa.*[27]

Risk factors for the acquisition of ESBL-producing *K. pneumoniae* by patients are: prolonged hospital stay; the use of broad spectrum antibiotics, especially cephalosporins; and the use of invasive devices.[9] These factors are usually the focus of investigation when an outbreak occurs. In our study, a HCW with onychomycosis worked in direct contact with patients in a neonatal intensive care unit and was persistently colonized with ESBL-producing *K. pneumoniae* identical with those obtained from clinical specimens from the patients. Onychomycosis may be a factor that maintains persistent colonization and needs to be taken into consideration in outbreak investigations.

REFERENCES

1. Mayhall CG. *Hospital Epidemiology and Infection Control.* 10th ed. Baltimore, MD: Lippincott Williams & Wilkins; 1996.
2. Richtmann R. *Diagnóstico e Prevenção de Infecção Hospitalar em Neonatologia.* Ed. Associação Paulista de Estudos e Controle de infecção Hospitalar; 2002.

3. Nouvellon M, Pons JL, Sirot D, Combe ML, Lemeland JF. Clonal outbreaks of extended-spectrum β-lactamase-producing strains of *Klebsiella pneumoniae* demonstrated by antibiotic susceptibility testing, β-lactamase typing, and multilocus enzyme electrophoresis. *J Clin Microbiol*. 1994;32:2625–2627.
4. Schiappa DA, Hayden MK, Matushek MG, et al. Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infection: a case-control and molecular epidemiologic investigation. *J Infect Dis*. 1996;174:529–536.
5. Gouby A, Neuwirth C, Bourg G, et al. Epidemiological study by pulsed-field gel electrophoresis of an outbreak of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in a geriatric hospital. *J Clin Microbiol*. 1994;32:301–305.
6. Shannon et al. Extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strains causing nosocomial outbreaks of infection in the United Kingdom. *J Clin Microbiol.* 1998;36:3105–3110.
7. Gastmeier P. A cluster of nosocomial *Klebsiella pneumoniae* bloodstream infections in a neonatal intensive care department: identification of transmission and intervention. *Am J Infect Control*. 2003;31:424–430.
8. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement*. M100-S14. vol 24 no. 1. Wayne, PA: Clinical and Laboratory Standards Institute; 2004.
9. Stürenburg E, Mack D. Extended-spectrum β-lactamases: implications for the clinical microbiology laboratory, therapy and infection control. *J Infection*. 2003;47:273–295.
10. Jacoby GA, Han P. Detection of extended-spectrum β-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. *J Clin Microbiol*. 1996;34:908–911.
11. Wolk D, Mitchell S, Patel R. Principles of molecular microbiology testing methods. *Infect Dis Clin North Am*. 2001;15:1157–1204.
12. Pfaller MA. Chromosomal restriction fragment analysis by pulsed-field gel electrophoresis. In: Isemberg HD, ed. *Clinical Microbiology Procedure Handbook*. Wasington, DC: American Society for Microbiology; 1993:10.
13. Parry MF, Hutchinson JH, Brown NA, Wu CH, Estreller L. Gram-negative sepsis in neonates: a nursery outbreak due to hand carriage of *Citrobacter diversus*. *Pediatrics.* 1980;65:1105–1109.
14. Rogues AM. Electronic ventilator temperature sensors as a potential source of respiratory tract colonization with *Stenotrophomonas maltophilia*. *J Hosp Infect*. 2001;49:289–292.
15. Sakhnini E. Fulminant *Stenotrophomonas maltophilia* soft tissue infection in immunocompromised patients: an outbreak transmitted via tap water. *Am J Med Sci,* 2002;323:269–272.
16. Melarned R. Successful control of an *Acinetobacter baumannii* outbreak in a neonatal intensive care unit. *J Hosp Infect* 2003;53:31–38.
17. Gaillot O, Maruejouls C, Abachin E, et al. Nosocomial outbreak of *Klebsiella pneumoniae* producing SHV-5 extended-spectrum-β-lactamase, originating from a contaminated ultrasonography coupling gel. *J Clin Microbiol*. 1998;36:1357–1360.
18. Vilar-Compte D. *Pseudomonas aeruginosa* outbreak in the area of surgical wound ambulatory care, in postmastectomy patients. *Salud Publica Mex*. 2003;45:371–378.
19. Hilton M, Chen JM, Barry C, Vearncombe M, Simor A. Deoxyribonucleic acid fingerprint in an outbreak of *Staphylococcus aureus* intracranial infection after neurotologic surgery. *Otol Neurotol*. 2002;23:550–554.
20. Weber S, Herwaldt LA, Mcnutt LA, et al. An outbreak of *Staphylococcus aureus* in a pediatric cardiothoracic surgery unit. *Infect Control Hosp Epidemiol*. 2002;23:77–81.
21. Wang JT, Chang SC, Ko WJ, et al. A hospital-acquired outbreak of methicillin-resistant *Staphylococcus aureus* infection initiated by a surgeon carrier. *J Hosp Infect*. 2001;47:104–109.
22. Meier PA, Carter CD, Wallace SE, Hollis RJ, Pfaller MA, Herwaldt LA. A prolonged outbreak of methicillin-resistant *Staphylococcus aureus* in the burn unit of a tertiary medical center. *Infect Control Hosp Epidemiol*. 1996;17:798–802.
23. Levin AS, Gobara S, Scarpitta CM, et al. Electric showers as a control measure for *Legionella* spp. in a renal transplant unit in São Paulo Brazil: Legionellosis Study Team. *J Hosp Infect*. 1995;30:33–37.
24. Matsuoka DM, Costa SF, Mangini C, Almeida GMD, Bento CN, Van der Heijden IN. A nosocomial outbreak of *Salmonella enteritidis* associated with lyophilized enteral nutrition. *J Hosp Infect.* 2004;58:122–127.
25. Donowitz L, Warsik F, Fisher K, Wenzel R. Contaminated breast milk: a source of *Klebsiella* bacteremia in a newborn intensive care unit. *Rev Infect Dis*. 1981;3:716–720.
26. Rogues AM, Boulard G, Allery A, et al. Thermometers as a vehicle for transmission of extended-spectrum-β-lactamase producing *Klebsiella pneumoniae*. *J Hosp Infect*. 2000;45:76–77.
27. Foca M, Jakob K, Whittier S, Della Latta P, et al. Endemic *Pseudomonas aeruginosa* infection in a neonatal intensive care unit. *N Engl J Med*. 2000;343:695–700.
28. Moolenaar RL, Crutcher JM, San Joaquin VH, et al. A prolonged outbreak of *Pseudomonas aeruginosa* in a neonatal intensive care unit: did staff fingernails play a role in disease transmission? *Infect Control Hosp Epidemiol*. 2000;21:80–85.
29. Gupta A, Della Latta P, Todd B, et al. Outbreak of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit linked to artificial nails. *Infect Control Hosp Epidemiol*. 2004;25:210–215.
30. Buxton AE, Anderson RL, Werdegar D, Atlas E. Nosocomial respiratory tract infection and colonization with *Acinetobacter calcoaceticus*: epidemiologic characteristics. *Am J Med*. 1978;65:507–513.
31. Adams BG, Marrie TJ. Hand carriage of aerobic Gram-negative rods may not be transient. *J Hyg (Lond)*. 1982;89:33–46.
32. Manning ML, Archibald LK, Bell LM, Banerjee SN, Jarvis WR. *Serratia marcescens* transmission in a pediatric intensive care unit: a multifactorial occurrence. *Am J Infect Control*. 2001;29:115–119.
33. Simhon A, Rahav G, Shapiro M, Block C. Skin disease presenting as an outbreak of pseudobacteremia in a laboratory worker. *Clin Microbiol*. 2001;39:392–393.

FETAL BREECH PRESENTATION PREDISPOSES TO SUBSEQUENT DEVELOPMENT OF SEPTIC ARTHRITIS OF THE HIP

Theodore J. Cieslak, MD, and Michael Rajnik, MD

Abstract: We postulated a link between breech presentation and septic arthritis of the hip. To examine the potential association between these 2 relatively uncommon entities, we used the Patient Administration Systems and Biostatistics Activity (PASBA) database, which contains coded information derived from hospitalizations and ambulatory encounters at U.S. military hospitals worldwide. Among a cohort of nearly 1 million infants born in a 14-year period, 3.37% were found to be in the breech presentation. The rate of septic arthritis of the hip or pelvis during the first year of life among these breech infants was ~1/8000, providing a relative risk of 4.1 (95% confidence interval, <1.4–11.7) compared with the rate among nonbreech infants. Fetal breech presentation predisposes to the development of septic hip during the first year of life.

Accepted for publication January 27, 2005.

From the San Antonio Military Pediatric Center, San Antonio, TX

Presented in part at the 42nd Annual Meeting of the Infectious Disease Society of America.[4]

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as necessarily reflecting the views of the Department of Defense or its individual component institutions.

Address for reprints: Col. Theodore J Cieslak, c/o Department of Pediatrics, Brooke Army Medical Center, Fort Sam Houston TX 78234. Fax 210-916-1740; E-mail Ted.Cieslak@amedd.army.mil.

DOI: 10.1097/01.inf.0000168851.56027.3f

Fetal breech presentation is associated with the orthopedic complications of neonatal hip instability[1] and congenital hip dislocation.[2] Experience with a neonate with concomitant breech deformation sequence and group B streptococcal septic arthritis of the hip

led us to question whether breech positioning in utero predisposed to the development of septic arthritis in the postnatal period. Given the relative rarity of septic hip (1.2–2.6/100,000 children) and of breech presentation (3–4%), a very large patient population would be needed to answer this question.

The Patient Administration Systems and Biostatistics Activity (PASBA) is an element of the U.S. Army's Medical Command in San Antonio, TX. Data management is a primary mission of PASBA, which maintains inpatient and ambulatory data records derived from hospital encounters at military facilities worldwide. The strength of PASBA lies in the very large numbers of patients potentially available through retrospective query. We used this PASBA database to examine the putative association between breech presentation and subsequent development of septic hip.

METHODS

Protocol approval was obtained through the Department of Clinical Investigation at Brooke Army Medical Center. All patient data relevant to this study were "deidentified"; informed consent was thus unnecessary.

Although partial inpatient records for Army hospitals contained within the PASBA database date back as far as 1964, we chose 1989 as a starting point for this retrospective study. It was in 1989 that a more thorough and standardized "Standard Inpatient Data Record" was implemented within the PASBA system. Moreover it was in 1989 that Air Force and Navy hospital data began to be included within the system. We ended the study in 2002 so as to have at least 1 full year of follow-up in all cases.

The PASBA database was queried electronically for the following Diagnosis Group (DG) Codes: V3x.x, live birth; 652.1, breech conversion; 652.2, fetal breech presentation; 669.6, breech extraction; 711.05, septic arthritis of the pelvis and thigh.

Records of mothers with DG 652.2 were cross-matched with those of children with DG 711.05 using the active duty service member's Social Security number. Although the database was also queried for DGs 652.1 and 669.6, the numbers of these conditions were too small to yield meaningful conclusions as to their independent effect on the subsequent development of septic arthritis. Breech conversion and breech extraction were therefore included among the totals in DG 652.2. Fetal presentations other than breech (face, brow, oblique, transverse) were not factored out from among the "all-others" group, most of whom would be expected to present in the vertex position. Rates of septic arthritis (DG 711.05) among children whose gestation was complicated by breech presentation were compared with those among children whose gestation involved any other presentation. Statistical analysis of relative risk with 95% Cornfield confidence intervals was conducted using the EpiInfo statistics package, version 6.04 days. Significance (defined as $P > 0.05$) was assessed with the Fisher exact test.

RESULTS

From January 1989 through September 2002, there were 949,483 live births at U.S. Army, Navy and Air Force hospitals throughout the world. Of these, 31,986 (3.37%) were complicated by fetal breech presentation. During the first year of life, 4 cases (0.012%) of septic arthritis subsequently occurred among the breech group, whereas 28 cases (0.003%) occurred among the 917,497 nonbreech infants. When those children old enough to have appropriate length of follow-up were studied to age 6 years, 197 cases of septic arthritis were noted among the nonbreech children, and 8 cases occurred in the breech group. Our results are summarized in Table 1. The relative risk of septic arthritis of the hip among children whose gestation involved breech presentation was 4.1 (95% confidence intervals, 1.4–11.7) during the first year of life. Relative risk was not increased beyond the first year of life.

DISCUSSION

The association between trauma and the subsequent development of septic arthritis or osteomyelitis at the site of injury has long been recognized. In infants, Edwards et al[3] noted a propensity for group B streptococcal osteomyelitis to involve the right proximal humerus and postulated that this might be a result of trauma caused by the passage of the shoulder beneath the maternal symphysis pubis during vaginal delivery. We theorized that the mechanical stress placed on the fetal hips and pelvis in the breech position might form the traumatic nidus that leads to a similar propensity for the development of septic arthritis of the hip. Our study lends support to this theory.

The study has several limitations, however. Our ability to access only those data entered into the database precludes a systematic examination of the microbial etiology of the infected hips. Our index patient (a 1-month-old) had infection caused by group B *Streptococcus*. A 1-year-old had infection caused by *Staphylococcus aureus,* and another 1-year-old had an unspecified infection after a case of varicella. The etiologic agents involved in the other 3 cases of septic hip occurring among breech infants during the first year of life (two 2-month-olds and an 11-month-old), as well as the pathogens involved in cases occurring in a 2-year-old and a 3-year-old, were either unknown or were not entered into the database.

PASBA data on inpatients are entered by International Classification of Diseases, 9th revision (ICD-9) codes. We postulated that it was fetal position rather than method of delivery that might predispose an infant to subsequently develop pathology of the hip. Because fetuses in the breech position might be delivered by breech extraction (an "infant code," coded in the newborn infant's record), breech conversion or caesarean section (both maternal codes), we elected to use DG 652.2 (breech presentation, a maternal code) to include all fetuses in this position late in gestation. Despite the

TABLE 1. Rates of Septic Arthritis of the Hip Among Breech and Nonbreech Infants During the First 6 Years of Life

Age (yr)	Total No.*	Nonbreech	Nonbreech + SA	Incidence/ 100,000	Breech	Breech + SA	Incidence/ 100,000	$P^{\dagger}$
0–1	949,483	917,497	28	3.1	31,986	4	12.5	0.02
1–2	895,645	865,305	58	6.7	30,340	2	6.6	NS
2–3	843,008	814,322	24	2.9	28,686	1	3.5	NS
3–4	788,461	761,529	38	5.0	26,932	1	3.7	NS
4–5	732,864	707,647	27	3.8	25,217	0	0	NS
5–6	674,670	651,353	22	3.4	23,317	0	0	NS

*Includes only those patients with adequate length of follow-up. Thus there are more patients in the 0–1-year age group than in the 5–6-year age group as the 5–6 group excludes anyone born during the last 6 years of the study.

†Two-tailed P value by Fisher's exact test.

SA indicates septic arthritis; NS, not significant ($P > 0.05$).

inclusion of nearly 1 million deliveries, the numbers of breech extractions and conversions were too small to permit assessment of the role of delivery method on the incidence of septic hip.

Within the military, medical records of all family members are coded and referenced using the Social Security Number (SSN) of the active duty service member "sponsor" (with an attendant prefix). This creates potential problems in the case of newborn infants. For example, an infant born to a single servicewoman would be referenced under this servicewoman's SSN. If it were later determined that another serviceman was the father, that record might be reassigned a new SSN. In such cases, linking a child's record with that of his/her mother would be difficult or impossible.

Furthermore the ICD-9 coding system has no specific code for septic hip. Rather this entity is coded as DG 711.05, septic arthritis of the pelvic region and thigh. Inclusion of conditions such as sacroiliitis would contaminate the data. Despite these limitations, which would likely affect both the breech and nonbreech groups equally, our data show a statistically relevant 4-fold risk of developing septic arthritis of the hip or pelvis during the first year of life among children whose in utero position involved a breech presentation. Although significant, the risk is low; even among breech infants, the risk of developing septic hip is only ~1/8000.

ACKNOWLEDGMENTS

We thank Terri Amrhein for her extensive assistance in searching and manipulating the PASBA database.

REFERENCES

1. Hinderaker T, Daltveit AK, Irgens LM, Uden A, Reikeras O. The impact of intrauterine factors on neonatal hip instability: an analysis of 1,059,479 children in Norway. *Acta Orthop Scand*. 1994;65:239–242.
2. Clausen I, Nielsen KT. Breech position, delivery route, and congenital hip dislocation. *Acta Obstet Gynecol Scand*. 1988;67:595–597.
3. Edwards MS, Baker CJ, Wagner ML, Taber LH, Barrett FF. An etiologic shift in infantile osteomyelitis: the emergence of the group B *Streptococcus*. *J Pediatr*. 1978;93:578–583.
4. Presented at the 42nd Annual Meeting of the Infectious Disease Society of America, October 2004, Boston, MA. Abstract 241.

HEPATITIS A VIRUS INFECTION PRESENTING WITH SEIZURES

Sebahat Cam, MD,† Deniz Ertem, MD,†
Ozge Altun Koroglu, MD, and Ender Pehlivanoglu, MD†*

Abstract: Hepatitis A infection rarely causes extrahepatic manifestations. Here we present a 5-year-old patient with an initial complaint of nuchal rigidity and convulsions during the course of hepatitis A infection. Because hepatitis A virus RNA was demonstrated in the cerebrospinal fluid, it was thought that convulsions might be related to this viral infection.

Key Words: hepatitis A, convulsion, extrahepatic, child, neurologic complication

Accepted for publication January 13, 2005.

From the *Department of Pediatrics and the †Division of Pediatric Gastroenterology, Hepatology and Nutrition, Marmara University School of Medicine, Istanbul, Turkey

Address for reprints: Ender Pehlivanoglu, MD, Division of Pediatric Gastroenterology, Hepatology and Nutrition, Marmara University School of Medicine, Tophanelioglu Cd. 13-15, 81190 Altunizade, Istanbul, Turkey. E-mail e.pehlivanoglu@tnn.net.

DOI: 10.1097/01.inf.0000168754.24478.6d

Although hepatitis A virus infection has usually a self-limited clinical course during childhood, it may occasionally be related to autoimmune and neurologic manifestations such as vasculitis, arthritis and meningoencephalitis.[1,2] Seizures are rarely associated with acute viral hepatitis. To our knowledge, 2 pediatric patients have been reported to have convulsions during the course of the disease.[3] A 5-year-old patient with an initial complaint of convulsion during the course of hepatitis A infection is presented. This is the first case in the literature in which hepatitis A IgM and viral RNA were detected in the cerebrospinal fluid (CSF).

CASE REPORT

A 5-year-old boy was admitted to the hospital because of generalized tonic-clonic convulsions. He had had 4 convulsions lasting 1–10 minutes in the last 24 hours. He complained of fatigue starting 1 week before admission. Past medical history was unremarkable with no history of head trauma, seizures or drug intake. There was no family history of febrile seizures or epilepsy.

On admission, the child was well-nourished and well-developed, awake and oriented. Physical examination revealed a nonfebrile child with slight jaundice. His liver was palpated 3 cm below the costal margin with mild tenderness over the right upper quadrant. There were no motor or sensory deficits on neurologic examination, and the only pathologic finding was the presence of nuchal rigidity. He was afebrile during the hospital stay.

Laboratory studies were normal regarding plasma concentrations of glucose, electrolytes, renal function tests, coagulation profile and complete blood cell count. Liver function tests were elevated (aspartate aminotransferase, 642 units/L; alanine aminotransferase, 1347 units/L; alkaline phosphatase, 620 units/L) with normal total protein and albumin values. Total serum bilirubin concentration was 7.6 mg/dL with a direct fraction of 7.1 mg/mL. Because the patient was icteric and had elevated liver function tests, the etiology of acute hepatitis was investigated. IgM and IgG antibodies to hepatitis A virus were detected in the serum of the patient. The serologies for other hepatotrophic viruses (hepatitis B virus, hepatitis C virus, Epstein-Barr virus, herpes simplexvirus and cytomegalovirus) were negative. Blood and urine cultures remained sterile. Serum ammonia, lactate and seruloplasmin values were also within normal limits.

Abdominal ultrasonography revealed increase in echogenicity of the liver parenchyma. Because the patient presented with acute seizures, a computerized tomographic scan of the central nervous system was performed to exclude trauma, hemorrhage and infarction. The magnetic resonance imaging of the brain was normal, and there were no signs of parenchymal abnormality or increased intracranial pressure. Electroencephalographic examination, performed on the next day of convulsions, was normal.

Because there was nuchal rigidity, a lumbar puncture was done to rule out a central nervous system infection. CSF was clear without any cells, the glucose concentration was 71 mg/dL and protein value was 20 mg/dL. The fluid culture was sterile. IgM for hepatitis A virus was positive in CSF. Further polymerase chain reaction examination of CSF for hepatitis A virus RNA was also positive.

DISCUSSION

Hepatitis A virus infection is usually a self-limited disease during childhood.[1,2] Autoimmune and neurologic manifestations such as cutaneous vasculitis, cryoglobulinemia, arthritis, glomerulonephritis, Guillain-Barré syndrome, radiculomyelopathy and meningoencephalitis were recently recognized.[2–4] Neurologic manifestations are rare during the course of acute hepatitis A infection.[5–7] The pathogenesis of seizures in acute viral hepatitis remains obscure. Disturbed detoxification process by the diseased liver and

accumulation of the toxic metabolic products is one proposed mechanism.[8] The second mechanism might be the direct invasion of the central nervous system by hepatitis A virus[9]; this has never been documented in humans. Immunologic mechanisms and activation of a latent neurotrophic virus by hepatitis A virus have been proposed but unproved at present.

Our patient was admitted to the hospital after having generalized tonic-clonic seizures. There was no history of previous convulsions. Predisposing factors such as head trauma, drug intake, fever or family history of epilepsy were excluded. He was awake, oriented and cooperative and his neurologic examination was unrevealing. Biochemical, serologic and metabolic investigations did not provide a reason for the seizures except the presence of hepatitis A virus in CSF. Because the other causes of convulsions were excluded, it was thought that the seizures might be related to hepatitis A virus infection. Detection of IgM antibody to hepatitis A virus in blood and CSF and viral RNA in the CSF supported our conclusion. During 2 years of follow-up of our patient, no further seizure activity or liver dysfunction has been detected. To our knowledge, this is the first patient in whom hepatitis A viral RNA has been detected in CSF and might provide an explanation for seizure activity.

Hepatitis A virus infection has a mild and self-limited course in childhood; nevertheless it should be added to the differential diagnosis of acute seizures if the liver enzymes are concomitantly elevated.

REFERENCES

1. Tong MJ, El-Farra NS, Grew MI. Clinical manifestations of hepatitis A: recent experience in a community teaching hospital. *J Infect Dis*. 1995; 171(suppl 1):S15–S18.
2. Willner IR, Uhl MD, Howard SC, et al. An analysis of patients hospitalized during an urban epidemic in the United States. *Ann Intern Med*. 1998;128:111–114.
3. Dollberg S, Hurvitz H, Reifen RM, et al. Seizures in the course of hepatitis A. *Am J Dis Child*. 1990;144:140–141.
4. Ertem D, Acar Y, Pehlivanoglu E. Autoimmune complications associated with hepatitis A virus infection in children. *Pediatr Infect Dis J*. 2001;20:809–811.
5. Thomas WJ, Bruno P, Holtzmuller K. Hepatitis A virus anicteric encephalitis coexistent with hepatitis C virus infection. *Am J Gastroenterol*. 1993;88:279–281.
6. Bosch V, Dowling P, Cook SD. Hepatitis A virus immunoglobulin M antibody in acute neurological disease. *Ann Neurol*. 1983;14:685–687.
7. Tabor E. Guillain-Barré syndrome and other neurologic syndromes in hepatitis A, B and non-A, non-B. *J Med Virol*. 1987;21:207–216.
8. Lucké B. Pathology of fatal epidemic hepatitis. *Am J Pathol*. 1944;20:471–593.
9. Weinstein L, Davison WT. Neurologic manifestations in the preicteric phase of infectious hepatitis. *Am Pract*. 1946;1:191–195.

CONGENITAL CYTOMEGALOVIRUS INFECTION DIAGNOSED BY POLYMERASE CHAIN REACTION WITH THE USE OF PRESERVED UMBILICAL CORD

Hiroko Kakizawa, MD, Akihisa Okumura, MA,* Yoshiko Suzuki, MD,* Jun Natsume, MD,† Hiroshi Kimura, MD,* Tamiko Negoro, MD,* and Kazuyoshi Watanabe, MD‡*

Abstract: We report a patient with congenital cytomegalovirus infection diagnosed retrospectively by real time polymerase chain reaction with the use of a preserved umbilical cord. The patient had mild developmental delay without apparent hearing loss at the diagnosis. Congenital cytomegalovirus infection was suspected on the basis of magnetic resonance imaging that showed abnormal signal intensities in the gray-white matter junction.

Key Words: congenital cytomegalovirus infection, neuroimaging, polymerase chain reaction, preserved umbilical cord

Accepted for publication January 13, 2005.

From the *Department of Pediatrics, Nagoya University Graduate School of Medicine, the †Depatment of Pediatrics, Nagoya First Red Cross Hospital, and the ‡Faculty of Medical Welfare, Aichi Shukutoku University, Nagoya, Japan

Address for reprints: Hiroko Kakizawa, MD, Department of Pediatrics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-Ku, Nagoya, Aichi 466-8550, Japan. Fax 81-52-744-2974; E-mail kakizawa@t-gh.bias.ne.jp.

DOI: 10.1097/01.inf.0000168842.56185.cd

The neurologic sequelae of congenital cytomegalovirus (CMV) infection differ depending on the timing of infection.[1] Infection in the third trimester can result in mental retardation and hearing loss in the infant. It is difficult to diagnose asymptomatic congenital CMV infections retrospectively after late infancy. Neuroimaging findings vary widely according to the timing of infection.[1–6] Normal gyral pattern, mild ventricular and sulcal prominence and white matter abnormalities are characteristic in patients infected near the end of gestation including in asymptomatic infants.[7]

Virologic proof is also difficult in patients with asymptomatic congenital CMV infection beyond neonatal period. However, there have been several reports on retrospective diagnosis of congenital CMV infection by polymerase chain reaction (PCR) for umbilical cord or blood stored on a Guthrie card.[8,9] We report a patient with a mild developmental delay caused by congenital CMV infection retrospectively proven by polymerase chain reaction with the use of preserved umbilical cord.

PATIENT REPORT

History. The patient was a 20-month-old girl at the first presentation. She was the first child of healthy nonconsanguineous parents. Although intrauterine growth retardation and oligohydramnion were recognized after 30 weeks of gestation, she was uneventfully born at 38 weeks of gestation by spontaneous delivery. Her Apgar score was 9 and 9 at 1 and 5 minutes, respectively. Her birth weight was 1816 g (−2.0 SD). Her neonatal course was unremarkable.

Her developmental milestones were mildly delayed. She achieved head control at 5 months of age, sat alone at 11 months and walked without support at 22 months. She spoke a word at 11 months of age and ~20 words at 18 months of age. The developmental quotient at 2 years 6 months of age was 78 by the new K form developmental test.[10] Brainstem auditory evoked potentials at 20 months of age were normal at 80 dB. However, follow-up brainstem auditory evoked potentials at 4 years of age revealed slightly abnormal interwave separation.

Magnetic resonance imaging (MRI) was performed at 21 months of age. Axial T_1-weighted images revealed multiple foci of low signal intensity in the gray-white matter junction. Axial T_2-weighted images showed abnormal high intensities in the same region as T_1-weighted images. Polymicrogyria or other cortical malformations were not observed.

Detection of CMV DNA. Detection of CMV DNA was performed by a real time quantitative PCR, as previously described,[11–14] with preserved umbilical cord. Briefly DNA was extracted from 5 mg of navel string using a QIAamp Blood Kit (Qiagen, Hilden, Germany). The real time PCR assay with a fluorogenic probe was performed with a TaqMan PCR kit and a Model 7700 Sequence Detector (Applied Biosystems, Foster City, CA). The amount of CMV-DNA was calculated and expressed as the number of virus copies per μg of DNA. From the patient's umbilical cord, 445 copies/μg DNA of

CMV DNA were detected. This result confirmed congenital CMV infection in the patient.

DISCUSSION

In our patient, MRI findings prompted us to consider congenital CMV infection. There have been several MRI studies on symptomatic and asymptomatic congenital CMV infection.[1–4] Infection before the 18th week of gestation leads to lissencephaly, whereas infection between the 18th and 24th weeks of gestation leads to focal dysplastic cortices. Normal cerebral cortices with delayed myelination are observed in infants infected during the third trimester. White matter lesions manifested as increased water are observed in children infected at any gestational age.[7] White matter changes are prominent in gray-white matter junctions and in periventricular zones. Characteristic white matter abnormalities in mildly retarded infants can be an indication of congenital CMV infection, although they are nonspecific.

Virologic diagnosis of congenital CMV infection is not difficult when an infant has characteristic symptoms at birth such as petechiae, hepatosplenomegaly, jaundice and microcephaly with calcification. Virologic confirmation is made by isolation of CMV from the urine or detection of CMV-DNA from the urine or blood in infants within 2 weeks after birth.[3] Detection of IgM CMV antibody is serologic proof. However, most asymptomatic infants will be missed during the neonatal period. Retrospective diagnosis of congenital CMV infection in a child can be difficult. It is a Japanese tradition to dry and store the umbilical cord of an infant. Our study showed that PCR with the use of umbilical cord is useful in retrospective diagnosis of congenital CMV infection, as reported in several previous reports.[15]

In conclusion, we proved congenital CMV infection by PCR with umbilical cord retrospectively in a patient with mild mental retardation whose MRI showed abnormal signal intensities in the gray-white matter junction without cortical abnormalities.

REFERENCES

1. Barkovich AJ, Lindan CE. Congenital cytomegalovirus infection of the brain: imaging analysis and embryologic considerations. *Am J Neuroradiol*. 1994;15:703–715.
2. Boesch C, Issakainen J, Kerwitz G, Kikinis R, Martin E, Boltshauser E. Magnetic resonance imaging of the brain in congenital cytomegalovirus infection. *Pediatr Radiol*. 1989;19:91–93.
3. Steinlin MI, Nadal D, Eich GF, Martin E, Boltshauser EJ. Late intrauterine cytomegalovirus infection: clinical and neuroimaging findings. *Pediatr Neurol*. 1996;15:249–253.
4. Gomes AL, Vieira JP, Saldanha J. Non-progressive leukoencephalopathy with bilateral temporal cysts. *Eur J Pediatr Neurol*. 2001;5:121–125.
5. Sugita K, Ando M, Makino M, Takanashi J, Fujimoto N, Niimi H. Magnetic resonance imaging of the brain in congenital rubella virus and cytomegalovirus infections. *Neuroradiology*. 1991;33:239–242.
6. Watemberg N, Vardi O, Lev D, Vinkler C, Lerman-Sagie T. Congenital cytomegalovirus infection presenting as an apparent neurodegenerative disorder. *Clin Pediatr (Phila)*. 2002;41:519–522.
7. Barkovich AJ. Infections of the nervous system. In: Barkovich AJ, ed. Pediatric neuroimaging. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:715–770.
8. Haginoya K, Ohura T, Kon K, et al. Abnormal white matter leasions with sensorineural hearing loss caused by congenital cytomegalovirus infection: retrospective diagnosis by PCR using Guthrie cards. *Brain Dev*. 2002;24:710–714.
9. Johansson PJ, Jonsson M, Ahlfors K, Ivarsson IA, Lars S, Guthenberg C. Retrospective diagnostics congenital cytomegalovirus infection performed by polymerase chain reaction in blood stored on filter paper. *Scand J Infect Dis*. 1997;29:465–468.
10. Ikuzawa M. *The New K Form Developmental Test* [in Japanese]. Kyoto, Japan: Nakanishiya Press; 1985.
11. Tanaka N, Kimura H, Iida K, et al. Quantitative analysis of cytomegalovirus load using a real-time PCR assay. *J Med Virol*. 2000;60:455–462.
12. Tanaka N, Kimura H, Hoshino Y, et al. Monitoring of four herpesviruses in unrelated cord blood transplantation. *Bone Marrow Transplant*. 2000; 26:1193–1197.
13. Yasuda A, Kimura H, Hayakawa M, et al. Evaluation of cytomegalovirus infections transmitted via breast milk in preterm infants with a real-time polymerase chain reaction assay. *Pediatrics*. 2003;111:1333–1336.
14. Hayashi N, Kimura H, Morishima T, Tanaka N, Tsurumi T, Kuzushima K. Flow cytometric analysis of cytomegalovirus-specific cell-mediated immunity in the congenital infection. *J Med Virol*. 2003;71:251–258.
15. Igarashi N, Yoshida T, Hatasaki K, Miyamori K, Morijiri Y, Miyagawa H. Unilateral lissencephaly due to congenital CMV infection: diagnosis with preserved umbilical cord tissue [in Japanese]. *Shonika Rinsho*. 2003;56:2000–2003.

PHOTOBACTERIUM DAMSELA BACTEREMIA IN A CHILD WITH SICKLE-CELL DISEASE

*Jennifer M. Knight-Madden, MB BS, FAAP,**
Michelle Barton, MB BS, DM,† Nischal Gandretti, MB BS,‡
and Alison M. Nicholson, MB BS, DM‡

Abstract: *Photobacterium damsela* was identified in a blood culture taken from a child with sickle-cell disease. This is the first report of this organism in humans in the Caribbean. The microbiology of this organism and its identification are discussed. The clinical presentation in humans and the role of the immune status of the patient are reviewed.

Key Words: sickle, *Photobacterium damsela*
Accepted for publication January 18, 2005
From the *Sickle Cell Unit, Tropical Medicine Research Institute, and the Departments of †Obstetrics, Gynaecology and Child Health and ‡Microbiology, University of the West Indies, Kingston, Jamaica, West Indies
Address for reprints: Dr Jennifer Knight-Madden, Sickle Cell Unit, TMRI, University of the West Indies, Mona, Kingston 5, Jamaica, West Indies
Fax 876-927-2984; E-mail jennifer.knightmadden@uwimona.edu.jm
DOI: 10.1097/01.inf.0000168845.26758.e3

Photobacterium damsela was first identified in 1981 as *Vibrio damsela* and later classified as *Listonella damsela* before being recently reclassified as *P. damsela* on the basis of phenotypic data. This organism got its name from the damselfish *Chromis punctipinnis*, perhaps its most common victim, which develops ulcers when infected with the bacterium. Like other vibrios, the organism is a halophilic Gram-negative, curved bacillus and is a natural inhabitant of the marine environment. The organism produces an exotoxin that is lethal in mice. This toxin is a cytolysin known as damselysin that appears to have strong hemolytic action on erythrocytes.[1] It is distinct from the toxins produced by some of the other vibrios such as *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio cholerae*.

Although it primarily infects fish, there have been rare reports of *P. damsela* being the cause of septicemia[2–4] and necrotizing fasciitis[5,6] in humans. Wound infection by *P. damsela* has been reported in 21 patients in English language journals to date, most of whom were from North America. This commonly follows exposure to brackish water or injury by saltwater animals.[7] *P. damsela* is a pathogen in immunocompromised and healthy hosts and can cause fulminant infections that are rapidly fatal even in immunocompetent hosts.

Although fishing is an important occupation in the Caribbean, there have been no reports of this pathogen in fish or humans. We

report the first case of *P. damsela* in the Caribbean in a child with sickle-cell disease, a disease that is known to impair the immune response.

CASE REPORT

S.W., identified by newborn screening as having sickle-cell disease, was followed from 1 month of age at the Sickle Cell Unit, the University of the West Indies. Penicillin prophylaxis was begun at 4 months of age, and the parents were compliant with this therapy. His course was unremarkable except for mild intermittent asthma and an episode of acute chest syndrome at 2 years 5 months of age.

At 2 years 5 months of age, he presented to a physician with a history of fever and dysuria for which trimethoprim-sulfamethoxazole (TMP-SMX) was prescribed. He remained unwell and thus presented to the University Hospital of the West Indies with a temperature of 39.3°C. Blood and urine were cultured with the results reported below. He was treated with amoxicillin/clavulanic acid, erythromycin, albuterol and oxygen. The albuterol and oxygen were discontinued when suspicion of an acute respiratory event was negated.

When a detailed history was obtained, it was learned that just before the child's illness, he had been handling fish brought home by his father who worked in a fish factory. At that time, he had an open right buttock wound which he scratched while handling the fish. Although the patient's wounds were healing well at the time of presentation and did not appear to be infected, it may have served as the portal of entry for the *P. damsela* organism. The organism would also have been eradicated by the antibiotics prescribed on presentation to the hospital.

Microbiologic Data. Blood received from the patient in one BacTec (Paed) bottle for culture and susceptibility testing was positive within 48 hours of incubation in the BacTec 9240 machine. A subsequent Gram stain done showed Gram-negative bacilli. The blood was then plated on to MacConkey agar and the non-lactose-fermenting bacteria that grew in 24 hours were identified and tested for susceptibility to antibiotics by the VITEK system (bioMérieux, Marcy-l'Etoile, France). The organism was identified as *P. damsela* and was susceptible to ampicillin, ceftriaxone, gentamicin and TMP-SMX and resistant to cefuroxime.

A significant growth of *Escherichia coli* was isolated from a catheter specimen of urine received from this patient. The organism was susceptible to a wide range of antibiotics including amoxicillin-clavulanic acid, noroxin and ceftriaxone and was resistant to ampicillin and TMP-SMX.

DISCUSSION

This is the first report of *P. damsela* infection in the Caribbean, although the organism has been found in warm ocean waters around the world.[8] The identification of this organism is likely valid as it occurred as a pure growth within *48 hours* and was identified by the BacTec system. Although the urinary tract infection was probably the major cause of the morbidity seen in this patient, the isolation of a pure growth of *P. damsela* in blood culture from this patient demonstrates that this organism is present in the local fish population and should be considered in humans who present with appropriate symptoms. This organism might not have been specifically identified before the introduction of the BacTec 9240 system in our laboratory in 2001. Molecular techniques such as polymerase chain reaction for the diagnosis of *P. damsela*[9] are unavailable locally.

Patients with sickle-cell disease are susceptible to infections by encapsulated organisms. *P. damsela* is sometimes encapsulated, with the encapsulated strains being more virulent. It is thus likely that this patient's sickle-cell disease status may have contributed to his infection with *P. damsela*. Immunocompetent persons may be affected by this organism.

It is difficult to determine the role that *P. damsela* played in this child's condition because there were comorbid conditions.

REFERENCES

1. Tison D. Vibrio. In: Murray P, Baron E, Pfaller M, Tenover F, Yolken R, eds. *Manual of Clinical Microbiology*. Washington, DC: ASM Press; 1999:497–506.
2. Perez-Tirse J, Levine JF, Mecca M. *Vibrio damsela*: a cause of fulminant septicemia. *Arch Intern Med*. 1993;153:1838–1840.
3. Shin JH, Shin MG, Suh SP, Ryang DW, Rew JS, Nolte FS. Primary *Vibrio damsela* septicemia. *Clin Infect Dis*. 1996;22:856–857.
4. Asato J, Kanaya F. Fatal infection of the hand due to *Photobacterium damsela*: a case report. *Clin Infect Dis*. 2004;38:e100–e101.
5. Coffey JA Jr, Harris RL, Rutledge ML, Bradshaw MW, Williams TW Jr. *Vibrio damsela*: another potentially virulent marine vibrio. *J Infect Dis*. 1986;153:800–802.
6. Goodell KH, Jordan MR, Graham R, Cassidy C, Nasraway SA. Rapidly advancing necrotizing fasciitis caused by *Photobacterium* (*Vibrio*) *damsela*: a hyperaggressive variant. *Crit Care Med*. 2004;32:278–281.
7. Blake PA. Vibrios on the half shell: what the walrus and the carpenter didn't know. *Ann Intern Med*. 1983;99:558–559.
8. Howard RJ, Bennett NT. Infections caused by halophilic marine *Vibrio* bacteria. *Ann Surg*. 1993;217:525–530.
9. O'Hara CM, Sowers EG, Bopp CA, Duda SB, Strockbine NA. Accuracy of six commercially available systems for identification of members of the family Vibrionaceae. *J Clin Microbiol*. 2003;41:5654–5659.

DETECTION OF HUMAN METAPNEUMOVIRUS FROM CHILDREN WITH ACUTE OTITIS MEDIA

Akira Suzuki, MD, Oshi Watanabe, MD,* Michiko Okamoto, BM,* Hiroko Endo, MD, PhD,† Hisakazu Yano, MD, PhD,‡ Mitsuko Suetake, MD, PhD,‡ and Hidekazu Nishimura, MD, PhD**

Abstract: Nasal and middle ear specimens collected from children with acute otitis media were subjected to viral isolation and bacteria culture. All virus-negative specimens underwent reverse transcription polymerase chain reaction to detect human metapneumovirus. Three of 126 middle ear specimens were positive by this assay.

Key Words: human metapneumovirus, acute otitis media

Accepted for publication January 3, 2005.

From the *Virus Research Center, Sendai Medical Center; and the Departments of †Pediatrics and ‡Otolaryngology, Tohoku Rosai Hospital, Sendai, Japan

Reprints not available.

DOI: 10.1097/01.inf.0000168755.01196.49

Studies have documented a close association between acute otitis media (AOM) and viral upper respiratory tract infection (URI) with respiratory syncytial virus (RSV), parainfluenza virus, adenovirus and influenza virus.[1] The occurrence of AOM shows extensive seasonal variation, and in temperate regions the incidence rates are highest during winter, which parallels the incidence of viral URI.[1]

The human metapneumovirus (hMPV), first isolated from children with URI in 2001,[2] may be a one of the major causative agents of URIs in young infants.[3] AOM is considered as a possible complication,[4–7] but these studies neither identified the bacterial pathogens of AOM nor examined microbiologically the middle ear fluid specimens. There has been no report of the detection of hMPV from patients with AOM. This study was aimed at the detection of hMPV from children with AOM.

METHODS

Between January 1 and May 31, 2003, a total of 138 children with AOM (94 bilateral AOM, 22 right AOM, 22 left AOM) were enrolled in the continuing study of the AOM in the Department of Otolaryngology, Tohoku Rosai Hospital. The diagnosis of otitis media was made on the basis of pneumatic otoscopic finding of fluid in the middle ear and also redness or bulging of the tympanic membrane. Of these patients, 81 were boys (59%) and 57 were girls (41%). Thirty-nine (28%) were younger than 12 months of age, 51(37%) were between 12 and 24 months of age, 20 (14%) were between 24 and 36 months of age, 25 (18%) were between 3 and 10 years of age and 3 (2%) were older than 10 years of age.

A middle ear fluid (MEF) specimen was obtained by tympanocentesis from one ear of each patient with either bilateral or unilateral AOM. Nasopharyngeal aspirate (NPA) specimens were collected from the nasopharynx through the nostrils. The specimens were sent immediately for bacterial culture to Tohoku Rosai Hospital or stored at 4°C in the clinic until they were transported to the Virus Research Center, Sendai Medical Center, within a few days of sampling. After arrival at the center, virus isolation was performed by microplate methods[8] with the use of 5 cell types (human embryo fibroblasts, HEp2, Madin-Darby canine kidney, Vero and HMV-II) subcultured on the 96-well microplates for the recovery of influenza viruses, parainfluenza viruses, RSV, adenovirus, rhinovirus and enterovirus. After inoculation of clinical samples onto cells, the cytopathic effects were examined daily for 2 weeks. Final identification of viruses was accomplished by standard neutralization tests with authentic antibodies or based on analyses of their genetic information.

NPA and MEF specimens that were virus culture-negative were tested by reverse transcription-PCR for the detection of hMPV. Briefly viral RNA was extracted from 100-μL samples with an RNeasy kit (Qiagen, Hilden, Germany) and complementary DNA was synthesized using 11 μL of eluted RNA with Moloney murine leukemia virus and random hexamer primers (Invitrogen, Carlsbad, CA). PCRs were designed to amplify the *N* or *F* gene of hMPV, and complementary DNA was amplified by a standardized PCR protocol with ExTaq (TaKaRa Bio, Otsu, Japan). The primer set used for the first PCR of the *N* gene were 5′-TTCAAGGATTCACCTGAGTG-3′ (N-1) and 5′-TCCTTAAGCAAGGTTGCCAT-3′ (N-2), and that for the nested PCR was N-1 and 5′-ACCACTTGGACTTCACTGCC-3′ (N-3).[9] The primer set for detection of the *F* gene was adopted from that described by Peret et al[10] The primer pair MPVF1f and MPVF1r, as described by Peret et al, was used for the first PCR of the *F* gene, and that for the nested PCR was MPVF1r and our original MVPF1f-2 (5′GTAAGAAGAAAAGGGTTCGG3′). We confirmed the results by direct sequencing of the PCR products with a Thermo Sequence Cy5.5 Terminator sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ) and an automated Gene Rapid sequencer (Amersham Pharmacia Biotech).

RESULTS

We isolated respiratory viruses from 36 (26%) of 138 NPA samples: 14 influenza viruses (9 influenza A/H3N2 viruses and 5 influenza B viruses); 7 adenoviruses; 4 RSVs; 4 parainfluenza viruses; 2 measles viruses; 2 cytomegaloviruses; and one each of enterovirus, rhinovirus and herpes simplex virus groups. Twelve (9%) of 138 MEF samples were virus-positive: 5 A/H3N2 influenza viruses; 2 adenoviruses; 2 measles viruses; and 1 each of RSV, enterovirus and rhinovirus. For each virus-positive MEF sample, the same type of virus was also isolated from NPA sample.

We cultured bacteria from 127 NPA samples (92%) and 70 MEF samples (51%) of 138 specimens. Of the 127 NPA samples, *Streptococcus pneumoniae* was detected in 15 cases, *Haemophilus influenzae* in 22, *Moraxella catarrhalis* in 7, *S. pneumoniae* and *H. influenzae* in 17, *S. pneumoniae* and *M. catarrhalis* in 26, *H. influenzae* and *M. catarrhalis* in 14 and other bacteria in 26, whereas in the 70 MEF samples, there were 25, 26, 5, 4, 0, 0 and 10 positive cases, respectively.

One hundred two NPA samples and 126 MEF samples that were virus-negative underwent reverse transcription-PCR for the detection of hMPV. Results were positive in 8 NPA samples and 3 MEF samples. The corresponding NPA samples of the 3 hMPV MEF samples were also positive for hMPV. Bacterial pathogens were found in the nasopharynges of 8 hMPV-positive cases, 7 being positive for *H. influenzae*, *S. pneumoniae* and/or *M. catarrhalis*. In 1 patient, patient A, hMPV was detected in the MEF and NPA samples, but a bacterial pathogen could not be cultured from the MEF sample (Table 1).

DISCUSSION

The detection of viruses in MEF samples of AOM is a central piece of evidence for a possible role of viruses in its pathogenesis, and we have detected hMPV from 3 MEF samples. The viral infection could cause congestion of the nasal and nasopharyngeal mucosa, resulting in Eustachian tube dysfunction which is an im-

TABLE 1. Detection of hMPV and Bacteria in NPA and MEF Specimens

Patient	Age (mo)	Sex	AOM	hMPV*		Bacteria†	
				NPA	MEF	NPA	MEF
A	20	F	Bilateral	+	+	*Haemophilus influenzae*	
B	11	M	Bilateral	+	+	*Streptococcus pneumoniae*	*S. pneumoniae*
C	40	F	Bilateral	+	+	*S. pneumoniae*/*Moraxella catarrhalis*	*S. pneumoniae*
D	17	F	Bilateral	+	−	*M. catarrhalis*	
E	24	F	Bilateral	+	−	*S. pneumoniae*/*M. catarrhalis*	
F	10	M	Bilateral	+	−	*S. pneumoniae*/*M. catarrhalis*	
G	5	M	Bilateral	+	−	*H. influenzae*	*H. influenzae*
H	5	M	Bilateral	+	−	*H. influenzae*/*S. pneumoniae*	*H. influenzae*

*Detection by nested PCR.
†Detection by culture methods.

portant factor in the pathogenesis of AOM.[1] In patient A, no bacterial or viral pathogen other than hMPV was detected in the MEF sample. Although we have not excluded other pathogens by alternative methods, this finding suggests the possibility that hMPV could be the sole pathogen for AOM. In contrast, hMPV was also detected from MEF samples of 2 cases in which *S. pneumoniae* was detected. The interaction of virus and bacteria in the pathogenesis of AOM is not fully understood. Animal studies suggests that viral infections confer a greater risk of AOM with colonization of *S. pneumoniae* and *H. influenzae* in the nasopharynx and worsen the clinical course of bacterial AOM.[1] Further studies are needed to clarify whether the interactions of hMPV with bacterial infections might have an effect on the inflammatory processes in the middle ear.

We used 2 diagnostic methods: nested PCR for hMPV; and viral isolation for other respiratory viruses. The PCR-based diagnostic techniques, especially nested PCR, heighten the sensitivity of the detection test. Furthermore the numbers of viruses were relatively low compared with those in other studies reporting the role of viruses in AOM.[1] Our study could have underdetected viruses, because only a virus isolation technique was used. Care must be taken in the comparison of the prevalence rates of hMPV with other viruses in our study.

Although, we could demonstrate that hMPV was associated with cases of AOM in children in winter and spring, we do not have definitive pathologic and microbiologic evidence to prove that hMPV can cause AOM.

ACKNOWLEDGMENTS

We thank Yukiko Watanabe, Izumi Tanaka, Hiroko Ito, Fumiko Chiba and Akira Ohmi for their assistance.

REFERENCES

1. Heikkinen T, Chonmaitree T. Importance of respiratory viruses in acute otitis media. *Clin Microbiol Rev*. 2003;16:230–241.
2. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med*. 2001;7:719–724.
3. van den Hoogen BG, Osterhaus ADME, Fouchier RAM. Clinical impact and diagnosis of human metapneumovirus infection. *Pediatr Infect Dis J*. 2004;23:S25–S32.
4. Peiris JS, Tang WH, Chan KH, et al. Children with respiratory disease associated with metapneumovirus in Hong Kong. *Emerg Infect Dis*. 2003;9:628–633.
5. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med*. 2004;350:443–450.
6. Boivin G, Abed Y, Pelletier G, et al. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. *J Infect Dis*. 2002;186:1330–1334.
7. Freymouth F, Vabret A, Legrand L, et al. Presence of the new human metapneumovirus in French children with bronchiolitis. *Pediatr Infect Dis J*. 2003;22:92–94.
8. Numazaki Y, Oshima T, Ohmi A, et al. A microplate method for isolation of viruses from infants and children with acute respiratory infections. *Microbiol Immunol*. 1987;31:1085–1095.
9. Suzuki A, Watanabe O, Miyabayashi S, et al. Epidemiological study of human metapneumovirus among pediatric patients with acute respiratory infection. *J Japan Pediatr Assoc*. 2004;36:390–394.
10. Peret TC, Boivin G, Li Y, Couillard M, et al. Characterization of human metapneumoviruses isolated from patients in North America. *J Infect Dis*. 2002;185:1660–1663.

ACUTE HYDROCEPHALUS CAUSED BY MUMPS MENINGOENCEPHALITIS

Handan Alp, MD, Hüseyin Tan, MD, Zerrin Orbak, MD, and Halil Keskin, MD

Abstract: Hydrocephalus is a rare complication of mumps. We report an 8.5-year-old boy with acute hydrocephalus associated with mumps meningoencephalitis.

Key Words: mumps, hydrocephalus, meningoencephalitis
Accepted for publication January 10, 2005.
From the Faculty of Medicine, Atatürk University, Erzurum, Turkey
Address for reprints: Handan Alp, MD, Atatürk University, Faculty of Medicine, Yakutiye Research Hospital, Department of Pediatrics, 25240, Erzurum, Turkey. Fax 90-442 236 13 01; E-mail handanalp@yahoo.com.
DOI: 10.1097/01.inf.0000168756.51518.99

The mumps myxovirus has a predilection for glandular and nervous tissues. Aseptic meningitis and/or encephalitis is a common manifestation of mumps infection.[1] Mumps meningoencephalitis causing acute hydrocephalus is a rare condition.[2–4] We report a child with mumps meningoencephalitis in whom hydrocephalus developed rapidly as a result of aqueductal stenosis.

CASE REPORT

On May 13, 2004, an 8.5-year-old boy was admitted to our clinic with the complaints of headache, vomiting and convulsion. He had been admitted to a local hospital with the diagnosis of mumps when bilateral parotid gland swelling appeared 10 days earlier. His local pediatrician referred him to our hospital because of "convulsive attacks, disoriented and semicomatose."

At the time of admission, the patient had a normal blood pressure and temperature. On neurologic examination, he was stuporous, responding to painful stimulation by opening his eyes and withdrawing. There was nuchal rigidity and Kerning and Brudzinski signs. Funduscopic examination, light reflex and deep tendon reflexes were normal. Glasgow Coma Scale was 10.

The cerebrospinal fluid (CSF) was clear, with protein of 214 mg/dL and pleocytosis of 200/mm^3 cell count (lymphocytes). IgM for mumps determined qualitatively in the patient's CSF and serum by enzyme-linked immunosorbent assay was positive. No bacterial microorganism grew from CSF cultures. An electroencephalogram revealed diffused abnormal slowing of the wave activity. A cranial computed tomography scan showed marked dilatation of the lateral and third ventricles with a normal fourth ventricle, without evidence of a space-occupying lesion. The patient was treated with antiedema therapy, and a ventriculoperitoneal shunt was placed. In the follow-up period of 8 weeks, the patient improved. When evaluated in September 2004, the patient had no neurologic deficit and had a normal school performance.

DISCUSSION

Mumps virus commonly invades the central nervous system, where it may produce no neurologic disease or initiate either aseptic meningitis or encephalitis. Depending on the criteria used for diagnosis, the incidence of meningitis or meningoencephalitis varies from a low figure of 10% to as high as 65%.[3] Moreover the finding of ependymal cell and cytoplasmatic inclusions of viral nucleocapsid-like material in the CSF of patients with mumps meningitis supports the hypothesis that mumps may cause granular ependymitis and, in rare instances, severe ependyma and subsequent aqueduct occlusion.[5–7]

In 1920, Dandy[8] was the first to suggest the role of inflammatory processes in the pathogenesis of aqueductal stenosis. Johnson et al,[9,10] first described the possible role of mumps viruses in the etiology of human hydrocephalus in suckling hamsters by intracerebral inoculation of mumps virus. Since then, 17 cases of hydrocephalus probably related to mumps virus infection have been reported in the literature.[2–4]

In the patients with meningitis or meningoencephalitis, the lag between mumps infection and hydrocephalus ranged from 24–48 hours to 19 years,[2,4] the latter seeming to be independent of the acute infectious event. This aspect can be deduced from the clinical cases of mumps previously published from the experimental studies on viral inoculations in rodents.[9,11]

In our case history, parotid gland swelling and a rise in IgM mumps antibody titer in the patient's serum and CSF strongly suggested mumps meningoencephalitis. Our patient's cranial computed tomography scan showed marked dilatation of the lateral and third ventricles with normal fourth ventricles, suggesting aqueductal stenosis. The patient was managed by performing a ventriculoperitoneal shunt.[4] In this patient, the time interval between mumps symptoms and acute obstructive hydrocephalus was 10 days, suggesting a rapid viral progression to the central nervous system.

REFERENCES

1. Maldonado Y. Mumps. In: Kliegman RM, Arvin AM, eds. *Nelson Textbook of Pediatrics*. 15th ed. Philadelphia, PA: W. B. Saunders Co., 2004;1035–1036.
2. Viola L, Chiaretti A, Castorina M, et al. Acute hydrocephalus as a consequence of mumps meningoencephalitis. *Pediatr Emerg Care*. 1998;14:212–214.
3. Timmons GD, Johnson KP. Aqueductal stenosis and hydrocephalus after mumps encephalitis. *N Engl J Med*. 1970;283:1505–1507.
4. Cinalli G, Spennato P, Ruggiero C, Alberti F, Maggi G. Aquaductal stenosis 9 years after mumps meningoencephalitis: treatment by endoscopic third ventriculostomy. *Child Nerv Syst*. 2004; 20:61–64.
5. Herndon RM, Johnson RT, Davis LE, Descalzi LR. Ependymitis in mumps virus meningitis: electron microscopical studies of cerebrospinal fluid. *Arch Neurol*. 1974;30:475–479.
6. Jellinger G. Anatomopathology of nontumoral aqueductal stenosis. *J Neursurg Sci*. 1986;30:1–16.
7. Sarnat HB. Ependymal reactions to injury: review. *J Neuropathol Exp Neurol*. 1995;54:1–15.
8. Dandy WE. The diagnosis and treatment of hydrocephalus resulting from strictures of the aqueduct of Sylvius. *Surg Gynecol Obstet*. 1920; 31:340–348.
9. Johnson RT, Johnson KP. Hydrocephalus following viral infection: the pathology of aqueductal stenosis developing after experimental mumps virus infection. *J Neuropathol Exp. Neurol*. 1968;27:591–606.
10. Johnson RT, Johnson KP, Edmonds CJ. Virus-induced hydrocephalus: development of aqueductal stenosis in hamsters after mumps infection. *Science*. 1967;157:1066–1067.
11. Takano T, Mekata Y, Yamano T, et al. Early ependymal changes in experimental hydrocephalus after mumps virus inoculation in hamsters. *Acta Neuropathol*. 1993;85:521–525.