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Absence of Infection in a Neonate after Possible Exposure to Sin Nombre Hantavirus in Breast Milk

In the spring of 1993, a cluster of patients with acute pulmonary disease associated with a high mortality rate was noted in the Four Corners region of the southwestern United States. This severe disease, termed hantavirus cardiopulmonary syndrome (HCPS), was found to be caused by a novel hantavirus, now known as Sin Nombre (SN) virus [1]. In Argentina and Chile, HCPS has been described in young children ranging in age from 2 to 11 years, and infants as young as 11 months of age have been infected with viruses associated with HCPS [2, 3]. In humans, maternal infection during gestation or in the perinatal period has not been associated with infection of the fetus or infant [4]. We report a case in which the possibility of perinatal transmission was unusually high. This case involved a mother who breast-fed her 3-week-old infant during the incubation period of HCPS.

On 1 July 1998, a 32-year-old woman developed fever, shortness of breath, and myalgia and presented to a rural health care facility for medical care. She had been breast-feeding her 3-week-old infant until a few hours before she presented for medical care. She was referred to the University Hospital in Albuquerque where her illness progressed to severe respiratory compromise. She acutely developed cardiac arrest and was treated with extracorporeal membrane oxygenation, which successfully stabilized her cardiopulmonary function; however, the period of cardiac arrest resulted in irreversible neurological damage, and she died on 22 July 1998. The infant was born on 9 June 1998; she was full term, and her birth was a spontaneous vaginal delivery. She had been healthy and growing appropriately before her mother's illness. She was exclusively breast-fed up until the onset of her mother's symptoms.

Blood samples were taken from the mother on 3 and 9 July 1998 and from the infant on 9 and 23 July 1998; EDTA was used for anticoagulation of these samples. Peripheral blood mononuclear cell (PBMC) and plasma fractions were prepared by use of density gradient centrifugation (Histopaque 1077; Sigma, St. Louis) as described elsewhere [5]. Breast milk samples were obtained from the mother on 3 and 9 July 1998. Breast milk from 9 July 1998 was separated immediately into cell and supernatant fractions by centrifugation.

Reverse transcriptase (RT)–PCR analysis detected SN virus RNA and confirmed the mother's infection [5, 6]. The nucleotide sequence of the RT-PCR product was determined from the PBMC fraction, and this sequence was typical for genomes of Four Corners region SN virus (data not shown) [5, 6]. Results of RT-PCR analysis of various blood and breast milk fractions are shown in figure 1. Both the mother's PBMC and plasma fractions were positive. In addition, unfractionated breast milk from 3 July 1998 was positive for SN virus RNA, indicating possible infectiousness at that time. The infant's PBMC and plasma fractions from 9 and 23 July 1998 were negative for SN virus RNA. All results of RT-PCR assay were confirmed by RT-PCR analysis with a different set of primers in the G2 gene (data not shown).

Antibodies to the SN virus were detected by a western blot

Informed consent was obtained from the patients or their parents or guardians, and the guidelines on human experimentation of the U.S. Department of Health and Human Services and the University of New Mexico Institutional Review Board were followed in the conduct of this research.

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Clinical Infectious Diseases 1999; 29:1577–9

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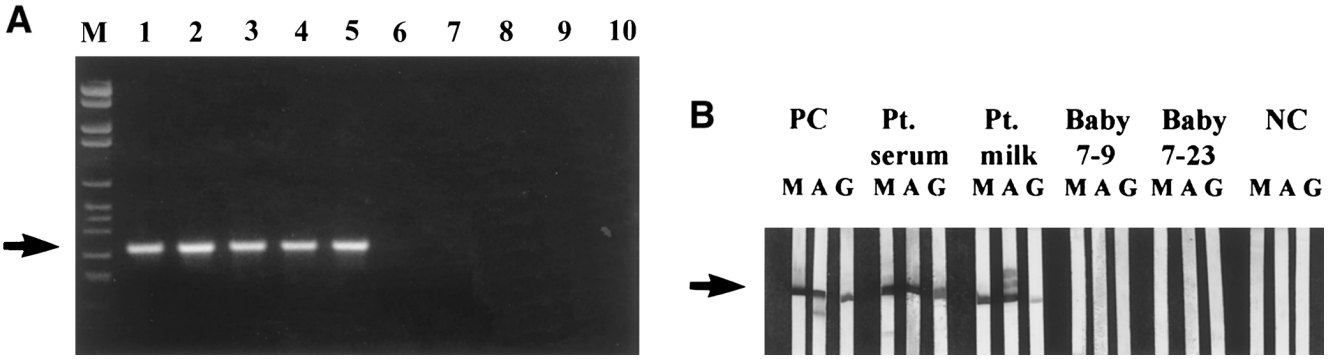

Figure 1. *A,* Results of reverse transcriptase (RT)–PCR analysis of breast milk and blood samples from a mother with Sin Nombre (SN) virus infection and of blood samples from her infant. Nested primers designed to detect the G1 gene of SN virus RNA were used. Arrow, migration of the 320-bp band of SN virus cDNA; M, molecular weight marker; lane 1, positive control; lane 2, patient's peripheral blood mononuclear cell (PBMC) fraction from 3 July 1998; lane 3, patient's serum sample from 3 July 1998; lane 4, patient's breast milk sample from 3 July 1998; lane 5, patient's breast milk cell fraction from 9 July 1998; lane 6, patient's breast milk supernatant fraction from 9 July 1998; lane 7, infant's PBMC fraction from 9 July 1998; lane 8, infant's PBMC fraction from 23 July 1998; lanes 9 and 10, negative controls. *B,* Results of western immunoblot assay of the mother's and infant's samples for the detection of antibodies to hantavirus. Each strip bearing recombinant-expressed nucleocapsid antigen of SN virus was incubated in the presence of serum or breast milk supernatant (1 : 200 dilution). Blots were then washed and incubated with goat antibody to human IgM (M), IgA (A), or IgG (G) conjugates. NC, negative control; PC, positive control; Pt., patient; 7-9, infant's serum sample from 9 July 1998; 7-23, infant's serum sample from 23 July 1998; arrow, migration position of the band of full-length recombinant nucleocapsid antigen.

assay with use of recombinant nucleocapsid antigen of SN virus [7, 8]. Results of western immunoblot assay of the samples are shown in figure 1. IgG, IgM, and IgA antibodies to the nucleocapsid antigen of SN virus were present in the mother's serum and breast milk. However, no antibodies to the nucleocapsid antigen of SN virus were detected in the infant's serum from either 9 or 23 July 1998. The infant was carefully monitored daily for fever and poor feeding but did not develop any discernible symptoms.

Neutralizing antibodies in the mother's serum and breast milk from 3 July 1998 were measured by using the focus reduction neutralization test (FRNT) described previously [9, 10]. FRNT of the mother's serum and breast milk from 3 July 1998 showed that both samples had significant titers of neutralizing antibodies ($\geq$1 : 640 and 1 : 320, respectively). By contrast, the baby's serum from 23 July 1998 lacked detectable neutralizing antibodies (titer, <1 : 10).

When we tested the mother's breast milk and the infant's blood from 9 July 1998, we realized that the infant had possibly been ingesting virus-contaminated milk; it showed no evidence of either passively transferred antibodies or a primary antibody response. There was a previous report suggesting that an infant might be at risk for infection through this type of exposure [2]. However, the infant did not develop symptoms, and there was no laboratory evidence of infection.

Therefore, the probable intake of SN virus–contaminated breast milk did not cause infection in the infant, nor were detectable maternal antibodies passed to the infant. Although the breast milk supernatant contained IgM, IgA, and IgG (a modest amount) antibodies to nucleocapsid antigen of SN virus, antibodies were not detectable in the infant's serum. The possibility remains that local neutralizing antibodies may have been sufficient to prevent viral transmission to the infant through the breast milk. However, we were not able to obtain samples of breast milk from the time the mother was breast-feeding the infant; therefore, we cannot be certain that either neutralizing antibodies or viral RNA was present at that time.

RT-PCR analysis, western blotting, and FRNT verified that the SN virus–infected mother did not transmit the infection to her infant through breast-feeding. However, the finding of SN virus RNA in the mother's breast milk during acute HCPS suggests that transmission could occur during this period. Perinatal transmission of SN virus could be influenced by a variety of factors including the disease stage, maternal viral load, and anatomic route (gastrointestinal, aerosol, or percutaneous) of exposure. Further studies are necessary to determine the possibility of postpartum transmission of SN virus infection and the role of protective maternal antibodies.

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Temporary Increase in Incidence of Invasive Infection Due to *Streptococcus pneumoniae* in the Netherlands

In 1996 and 1997, the Netherlands Reference Laboratory for Bacterial Meningitis (Amsterdam) noted an increase in *Streptococcus pneumoniae* isolates from blood but not from CSF [1]. To find an explanation for this increase, we determined the incidence of invasive pneumococcal disease detected in the period 1991–1998 by 6 regional public health laboratories covering 18% of the Dutch population (15.4 million inhabitants in 1994). Subsequently, we related this incidence to the incidence of influenza and the severity of the winters in the study period [2].

Invasive pneumococci were defined as those isolated from blood or CSF. Repeated isolation of invasive *S. pneumoniae* from the same patient within 1 month was ignored. Incidences were calculated with 1 August as the start of a "pneumococcal year" (i.e., the year 1997 was from 1 August 1996 to 31 July 1997). The Royal Meteorological Institute (KNMI, De Bilt, Netherlands) provided a winter severity index, calculated as the negative sum of the negative 24-h average temperatures (°C) in the period 1 November to 31 March. The incidence of influenza-like illnesses was derived from a countrywide network of general practitioners (NIVEL, Utrecht, Netherlands). Age-specific incidences were calculated by use of yearly updated demographic data and the postal codes of the patients in the areas served by the laboratories. Differences in incidence between years and contributing laboratories were tested simultaneously by means of PROC GENMOD of SAS Version 6.12 (SAS Institute, Cary, NC), with year and laboratory as classes, the number of blood cultures as a covariate, and the catchment population as the offset variable.

During the study period, 2182 *S. pneumoniae* isolates from blood and 113 *S. pneumoniae* isolates from CSF, summarized as invasive isolates, were reported by the participating laboratories. A second invasive isolate of *S. pneumoniae* was recovered only from 9 patients. Therefore, the number of confirmed cases of invasive pneumococcal infection and the number of invasive pneumococcal isolates were equal.

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Table 1. Winter severity index, incidence of influenza-like illnesses, no. of cases of invasive pneumococcal disease (IPD) per 1000 blood cultures, and incidence of IPD in the Netherlands by year.

Year	Winter severity index[a]	Incidence[b] of influenza-like illnesses	No. of cases of IPD/1000 blood cultures	Incidence[b] of IPD by age group (y)				
				Total	0–1	2–29	30–64	≥65
1991	77	2590	NA	8.6	19.7	2.8	5.2	36.1
1992	34	3170	6.0	8.3	29.6	1.6	5.3	34.2
1993	41	2950	6.3	9.2	17.0	2.8	5.7	38.5
1994	63	2960	5.9	8.4	15.7	1.6	5.2	37.5
1995	22	2060	6.8	10.9	33.0	2.3	8.2	40.3
1996	151	2630	9.3	14.8	18.7	2.6	10.4	63.4
1997	132	2264	8.1	13.3	31.9	3.0	8.7	53.7
1998	19	1970	5.1	8.9	15.9	1.4	5.7	39.1
1991–98		2574	6.8	10.3	22.7	2.3	6.9	43.1

NOTE. Catchment populations in 1994 for age groups were as follows: total, 2,771,992; 0–1 year, 70,177; 2–29 years, 1,045,229; 30–64 years, 1,291,126; and ≥65 years, 365,460. NA, not available.
[a] Negative sum of negative 24-h average temperatures.
[b] No. of cases per 100,000 person-years.