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Pseudo-Outbreak of Adenovirus Infection in a Neonatal Intensive Care Unit Due to a False-Positive Antigen Detection Test▽

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Twenty-eight of 56 infants in a neonatal intensive care unit had stools positive for adenovirus by the Sure-Vue adenovirus test. Virus cultures of conventionally processed and chloroform-extracted stool samples, as well as conventional and real-time PCR tests, were negative for adenovirus. The cause for the 50% false-positive rate with the antigen test was not determined.

On 11 September 2009, an infant with increased stool output was diagnosed with adenovirus gastroenteritis by the Sure-Vue adenovirus test (SA Scientific, San Antonio, TX). The index case was a 47-day-old, 30-week gestational female, whose problems included jejunal atresia that had been repaired surgically, apnea and bradycardia of prematurity, and hyperbilirubinemia. At the time of the false-positive antigen test, she was feeding well and exhibited abnormally frequent watery stools with a mild loss of weight. Three of five other infants in the same room were tested and found to be positive. Stool specimens from all infants in the neonatal intensive care unit (NICU) were collected, transported to the laboratory without viral transport media, and refrigerated prior to testing. No samples in diapers were sent to the laboratory. All specimens were tested within 12 h of collection; most were tested within 8 h of collection. Stool specimens from all 56 infants in the NICU were tested, and 28 were positive for adenovirus antigen. The positive reactions occurred within 15 min, as specified by the manufacturer. All of the reactions were graded 1+ to 2+ on a scale of 0 to 4+. All positive reactions gave a pink or red band like the control. The entire NICU was placed on contact precautions, and all health care workers were required to wear gowns and gloves when caring for the infants.

Table 1 compares the characteristics of the 28 adenovirus-positive and the 28 adenovirus-negative infants. The two groups were similar with respect to gender, chronologic age, gestational age, weight, weight loss in the preceding day, and antibiotic exposure, and similar numbers in the two groups had experienced emesis, necrotizing enterocolitis (NEC), gastrointestinal (GI) surgery, and poor feeding. Five infants (18%) in the antigen-positive group exhibited increased stool output in the day prior to the test compared to none in the antigen-negative group ($P = 0.03$).

Additional studies were conducted on 13 of the antigen-positive stools; these 13 specimens included those from the five infants with increased stool output. Stools were homogenized and centrifuged, and the supernatant was dispersed into two aliquots. One aliquot was mixed 1:1 with chloroform. Chloroform and the interface were removed, and the remaining aqueous portion was left open to allow residual chloroform evaporation. Both aliquots (untreated and chloroform-treated supernatant) were inoculated into separate tubes of A-549 cells. The cells were incubated for 14 days and examined 3 times per week for cytopathic effects. All cultures were negative for adenovirus. The same 13 specimens were also prepared for PCRs. The primers for conventional PCR, designed by Okada et al. (6), amplified a 950-bp region of the hexon gene with modified conditions. The real-time PCR utilized primers and a probe designed by Heim et al. (5) and targeted the hexon gene in a different location than did the conventional PCR. All PCR tests were negative for adenovirus DNA.

These results demonstrated that the positive antigen tests produced by the Sure-Vue adenovirus test were falsely positive. Suspicions about the validity of the positive antigen reactions were first raised when the number of ill infants proved to be limited to 5 of the 28 positive cases. Additionally, freezing stool specimens at −70°C for 24 h followed by thawing reduced the positivity rate 73%. Since adenovirus is nonenveloped and not particularly sensitive to such environmental changes, the disappearance of positive reactions upon freezing and thawing the specimens suggested that the original positive reactions were false. The failure to detect evidence of adenovirus by rigorous culturing methodology or by two PCR methods that target different conserved genomic sites established the absence of adenovirus in the antigen-positive specimens. Parenthetically, it should be noted that the Sure-Vue adenovirus test is an immune chromatographic test that utilizes a pair of adenovirus-specific monoclonal antibodies directed toward the hexon protein.

The reason for the false-positive reactions in stool specimens from infants hospitalized in a NICU was not determined. False-positive test results have been seen with *Staphylococcus aureus* strains that possess large amounts of protein A (4). None of the infants with positive antigen tests in this report were known to harbor such organisms; however, stool specimens were not cultured specifically for these bacteria. The consequences of the pseudo-outbreak were severe. The need to use gowns and gloves throughout the NICU for 2 months proved costly and created barriers to the care of the infants.

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TABLE 1. Characteristics of adenovirus antigen-positive and -negative infants

Characteristic	Value for infants who were:	
	Antigen positive	Antigen negative
No. of infants	28	28
Gender ratio (male:female)	15:13	18:10
Mean chronological age (days)	45	31
Mean gestational age (wk)	31	33
Mean wt (kg)	2.517	2.819
No. experiencing wt loss	8	4
No. experiencing emesis	2	2
No. experiencing NEC	1	0
No. experiencing GI surgery	7	2
No. with antibiotic exposure	7	10
No. with poor feeding tolerance	1	5

Adenovirus outbreaks in the nursery setting have been reported previously and have proven costly in terms of morbidity and mortality (1–3, 7). Finn et al. (3) reported an outbreak of adenovirus 7a in two neonatal units involving 9 infants, 10 staff members, and 3 parents. The intensive care nursery had to be closed to admissions for 19 days, and the second nursery closed for 14 days. Seventeen infants had to be transferred to other hospitals. In 1992 Piedra et al. (7) reported an outbreak of adenovirus type 8 among 11 of 112 infants in a neonatal intensive care nursery. The adenovirus caused severe respiratory disease requiring prolonged respiratory support and prolonged hospitalization. This report was followed in 1993 by a second report of an adenovirus type 8 outbreak in another neonatal intensive care unit in which four of seven premature infants who had undergone ophthalmic examination developed severe conjunctivitis (1). Subsequently, 16 adults and 6 additional children developed bilateral conjunctivitis. The important role that ophthalmologic examination has played in adenovirus outbreaks cannot be overly emphasized. Adenovirus type 30 was introduced into our own neonatal intensive care unit through eye examination, with devastating effects (2). A total of 21 infants acquired the infection, and 5 developed severe pneumonia and died. Thus, detection of adenovirus in the nursery setting is a serious occurrence that requires immediate and thorough investigation. The methods used to detect adenovirus must be reliable. The Sure-Vue adenovirus antigen test should not be used in a NICU setting until the mechanism of the false-positive reaction is elucidated.

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