Outbreak of Neonatal Gastroenteritis Associated with Astrovirus Serotype 1 at a Hospital in Inner Mongolia, China

Chun-ying Li,† Na Liu,2† Wei-dong Guo, Qiong Yu, Wen-rui Wang, Zhuang-Zhi Song, Hai Yan, Yun Luo, Ai-tao Lu, Hui-Ying Li, Lin Zhu, and Zhao-jun Duan†*

Inner Mongolia Autonomous Region Centre for Disease Control and Prevention and State Key Laboratory for Molecular Virology and Genetic Engineering, National Institute for Viral Disease Control and Prevention, China CDC, Beijing 100052, China

Received 20 April 2010/Returned for modification 23 June 2010/Accepted 23 August 2010

This report describes for the first time an outbreak of acute gastroenteritis among neonates associated with human astrovirus (HAstV) serotype 1b at a maternity hospital in Inner Mongolia, China. Of 40 specimens, 28 were astrovirus positive and rotavirus, calicivirus, and adenovirus negative. Poor hygiene likely contributed to the spread and persistence of HAstV in the neonatal care room.

Human astrovirus (HAstV) is a common cause of childhood diarrhea, especially in those less than 2 years old. Gastroenteritis outbreaks associated with HAstV infection have been reported in children’s day care centers (4, 7, 10) and schools (12) as well as in care centers for the elderly (9). HAstV infection usually results in mild disease, but outbreaks often involve a high number of children (8). Mixed infection of HAstV with rotavirus, norovirus, and adenovirus has often been reported (7). HAstV was first described in 1975 during an outbreak of diarrhea in the nursery of a maternity ward, but few such reports have been published subsequently, one example being a study from Thailand (14).

HAstVs are classified into eight serotypes according to the reactivity of the capsid proteins with type-specific monoclonal antibodies. HAstV1 is the most prevalent strain globally, HAstV2 to HAstV4 less so, and HAstV5 to HAstV8 the least prevalent (11). Recombination of HAstVs is seldom reported. Walter et al. characterized a HAstV3/5 recombinant strain and located a potential recombination site at the ORF1b/ORF2 junction (15).

In this study, we describe for the first time an outbreak among neonates of gastroenteritis associated with HAstV1 at a maternity hospital in Inner Mongolia, China. Diarrhea in neonates is defined on the basis of increased frequency and watery consistency of stools compared with their regular pattern. From 9 October 2008 to 13 February 2009, 61 neonates born in the hospital developed diarrhea. Over the following 4 months, a total of 61 neonates born in the hospital developed diarrhea while in the hospital or within 7 days of discharge. The outbreak incidence curve is shown in Fig. 1. From the eighth week of the outbreak to week 18, the incidence increased rapidly, with the exception of week 14. The final case was reported during week 19.

Fecal specimens were obtained from 40 subjects and stored at −70°C until required. Fecal suspensions (10%) were screened for group A rotavirus, adenovirus, and astrovirus using the IDEIA rotavirus, adenovirus, and astrovirus kits (Dako Diagnostics Ltd., Glostrup, Denmark), respectively. Multiplex reverse transcriptase-PCR (RT-PCR) and PCR were performed for the detection of norovirus GI, GII, sapovirus, astrovirus, and adenovirus in accordance with a previously published protocol (13). Purified PCR products were sequenced by Invitrogen. The resulting sequences were analyzed by CLUSTAL X (version 1.83) software followed by phylogenetic analysis using MEGA (version 4.1).

All 40 stool specimens were rotavirus, calicivirus (norovirus GI, GII, and sapovirus), and adenovirus negative by enzyme-linked immunosorbent assay (ELISA) and/or RT-PCR. However, HAstV was detected in 28 of 40 specimens (70%) by RT-PCR and 22 of 35 (62.86%) by ELISA. Furthermore, seven HAstV-positive specimens were subjected to electron microscopic (EM) examination; particles with the typical HAstV star form were observed in each.

PCR products from 13 HAstV-positive specimens were sequenced. BLAST analysis showed that 12 isolates had a high
level of homology (98%) to a genotype 1 HAstV, WH247, while another had 99% homology with the Melb1E strain. Furthermore, based on phylogenetic analysis of a 348-bp region of the HAstV ORF2 gene, HAstV-1s could be classified into four lineages (HAstV1a to -1d). All strains in this study clustered into lineage 1b (Fig. 2); they had 97.3% to 100% homology to each other and a sequence variation compared to other HAstV-1 lineages of between 8.6% and 11.2%. Two samples (NM58951 and NM58981) had 100% amino acid sequence identity to the Melb1E reference strain; the remainder differed at residue 188 (Lys replaced with Arg).

In astrovirus-positive samples, PCR was performed to am-
plify the 289-bp ORF1a and 1,260-bp ORF1b/ORF2 regions using primers Mon340/Mon348 and Mon344/Mon270, respectively. Reaction conditions were as described previously (15). Nucleotide sequences were obtained for ORF1a from 13 and for the ORF1b/ORF2 junction region from 3 of 28 HASV-positive samples. Sequence analysis of ORF1a and ORF1b/ORF2 showed 90.8% to 91.7% and 91.7% to 92.0% identities, respectively, to the prototype HASV-1 strain (GenBank no. L23513). Phylogenetic analysis suggested that all sequences clustered into the same branch of the HASV-1 strain.

Clinical symptoms observed in the outbreak were as follows. Fever was observed in a few patients (n = 4; range, 37.7°C to 38.4°C). No vomiting was noted in any subject. The mean duration of diarrhea was 2.79 days (95% CI, 2.27 to 3.31; range, 1 to 10 days). The mean frequency of loose stools was 7.95 per day (95% CI, 7.38 to 8.52; range, 4 to 11/day). Of the 61 subjects, 15 (24.6%) had underlying conditions, broken down as thrush (n = 5), pneumonia (n = 2), premature birth (n = 4), hyperbilirubinemia (n = 2), intrauterine infection (n = 1), and harelip/cleft lip and cerebral hemorrhage (n = 1). The mean duration of hospitalization was 10.18 days (95% CI, 8.74 to 11.63 days). Of the 40 subjects from whom stool specimens were tested, the mean duration of diarrhea was 2.79 days (95% CI, 1.90 to 3.59 days) in 28 astrovirus-positive cases and 2.75 days (95% CI, 1.84 to 3.66 days) in 12 astrovirus-negative cases. The mean frequencies of loose stools were 7.35 per day (95% CI, 6.58 to 8.12/day) and 8.25 per day (95% CI, 6.28 to 9.57/day) in HASV-positive and -negative subjects, respectively. Of the 28 positive subjects, 7 had underlying conditions, and there were 3 in the 12 negative subjects. The mean duration of hospitalization was 9.36 days (95% CI, 7.58 to 11.14 days) and 11.00 days (95% CI, 8.16 to 13.84 days) in HASV-positive and -negative subjects, respectively. No significant differences in the clinical symptoms between the groups were observed.

Outbreaks of diarrhea due to human astrovirus have frequently been reported worldwide (1, 7) and typically associated with HASV-1, HASV-2, and HASV-3. However, reports of gastroenteritis outbreaks among neonates are rare. In the present study, rotavirus, calcivirus (noroviruses G1 and GII and sapovirus), and adenovirus were not detected by either ELISA or RT-PCR in any of the 40 stool specimens. However, 28 (70%) were HASV positive by RT-PCR and corroborated by the ELISA data. These data suggest strongly that this outbreak was caused by HASV.

HASV-1 is the most prevalent serotype circulating globally, as well as the most prevalent HASV serotype reported in previous studies from China (6). In the present study, a comparison of ORF1a and ORF1b/ORF2 nucleotide sequences suggested that the Inner Mongolia strains were not recombinant and belonged to HASV serotype 1. Furthermore, based on the phylogenetic analysis of the 348-bp region of the HASV ORF2 gene, they all clustered into lineage 1b. This is in accordance with other reports from China (6). All these data suggest that the outbreak was caused by lineage 1b of human astrovirus (HASV) serotype 1 without recombination between ORF1 and ORF2, and they indicate the importance of HASV-1b in China.

Astroviral diarrhea has been regarded as being shorter in duration and of less severity than that caused by other enteric viruses (2, 3), but young children are considered to have more severe disease. In the present outbreak, the fact that no vomiting was reported and that few subjects were febrile did not mean the symptoms caused by astroviral diarrhea were mild. The frequency of diarrhea was high, with a mean of 7.95 episodes per day. The mean duration of hospitalization was more than 10 days. Taken together, these findings suggest that HASV can cause severe diarrhea among neonates.

The outbreak lasted more than 4 months. HASV incidence peaked twice during the outbreak (Fig. 1), which suggests that HASV persisted in the environment during the outbreak and in this way infected previously healthy subjects. Indeed, previous studies have reported that astrovirus can persist for approximately 2 months on contaminated surfaces (5). Persistence of astrovirus on contaminated surfaces within the neonatal care room may explain the lengthy duration of the outbreak.

Poor hygiene in the neonatal care room likely contributed to the spread and persistence of HASV. A neonate born in the hospital, after close postnatal observation for approximately 6 h, is usually transferred to the maternity ward and is “roomed” with its mother. During the first 6 h of life, milk was fed to neonates at least once with reusable feeding bottles. The feeding bottles were washed only in clean water prior to the next use. Also, neonates were bathed at least once, but bath water was changed only after three or four children were bathed. These factors in all probability enhanced person-to-person transmission and put the neonates at high risk of HASV infection. A lack of environmental samples from the neonatal care room meant that direct evidence of the relationship of infection and environmental persistence of HASV was not obtained.

This study is to our knowledge the first to link a particular HASV serotype (HASV-1b) to an outbreak of diarrhea among neonates in a maternity hospital. Hygienic practices by the staff of the neonatal care room should be improved to prevent further outbreaks. In addition, surveillance should be enhanced to better understand the role of HASV in outbreaks of gastroenteritis and provide more information on the extent of HASV infection among young children.

**Nucleotide sequence accession numbers.** The ORF2, ORF1a, and ORF1b/ORF2 nucleotide sequences were deposited in GenBank under accession numbers GU363516 to GU363528, HM060956 to HM060968, and HM120876 to HM120878, respectively. The sequences of reference strains were obtained from GenBank for comparison with sequences obtained in this study.

This work was partly supported by the China Mega-Project for Infectious Disease (2009ZX10004-001) and the National High Technology Research and Development Program of China (grant no. 2006AA02A215).

None of us has a conflict of interest.

**REFERENCES**


