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

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This report describes for the first time an outbreak of acute gastroenteritis among neonates associated with human astrovirus (H AstV) 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 H AstV in the neonatal care room.

Human astrovirus (H AstV) is a common cause of childhood diarrhea, especially in those less than 2 years old. Gastroenteritis outbreaks associated with H AstV 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). H AstV infection usually results in mild disease, but outbreaks often involve a high number of children (8). Mixed infection of H AstV with rotavirus, norovirus, and adenovirus has often been reported (7). H AstV 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).

H AstVs are classified into eight serotypes according to the reactivity of the capsid proteins with type-specific monoclonal antibodies. H AstV1 is the most prevalent strain globally, H AstV2 to H AstV4 less so, and H AstV5 to H AstV8 the least prevalent (11). Recombination of H AstVs is seldom reported. Walter et al. characterized a H AstV3/5 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 H AstV1 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 while in the hospital or within 7 days of discharge. The outbreak incidence curve is shown in Fig. 1. Fecal specimens were obtained from 40 subjects and stored frozen 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).

Of a total of 61 subjects, 42 (68.8%) were male and 19 (31.2%) were female (male-female ratio, 2.2:1). The mean age of subjects was 7.3 days (95% confidence interval [CI], 6.4 to 8.3 days). Of 28 H AstV-positive subjects, 21 (66.7%) were male and seven (33.3%) were female (male-female ratio, 3:1) and their mean age was 7.0 days (95% CI, 5.4 to 8.6 days). H AstV detection rates in male and female subjects were both 70.0% (21 of 30 and 7 of 10, respectively). H AstV was detected in 18 of 25 (72.0%) and 10 of 15 (66.7%) of subjects aged 7 or fewer days and more than 7 days, respectively. This difference was not significant.

The epidemic began on 9 October 2008, when a neonate (born 24 September 2008) 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.

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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, H AstV was detected in 28 of 40 specimens (70%) by RT-PCR and 22 of 35 (62.86%) by ELISA. Furthermore, seven H AstV-positive specimens were subjected to electron microscopic (EM) examination; particles with the typical H AstV star form were observed in each.

PCR products from 13 H AstV-positive specimens were sequenced. BLAST analysis showed that 12 isolates had a high
level of homology (98%) to a genotype 1 HAstV, WH2447, 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-

![FIG. 1. Incidence curve of diarrhea among neonates at a hospital in Inner Mongolia, China, from 9 October 2008 to 13 February 2009.](image)

![FIG. 2. Phylogenetic analysis of a 348-bp region of HAstV ORF2 (capsid region) amplified from 13 stool samples. The tree was constructed using the neighbor-joining method, and the numbers on the branches indicate the bootstrap values. GenBank accession numbers of the porcine astrovirus (PAstV) outgroup and reference strains of HAstV are given in parentheses.](image)
plit 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 HAstV-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 HAstV-1 strain (GenBank no. L23513). Phylogenetic analysis suggested that all sequences clustered into the same branch of the HAstV-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 = 6), pneumonia (n = 2), prematurity 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 HAstV-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 HAstV-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 HAstV-1, HAstV-2, and HAstV-3. However, reports of gastroenteritis outbreaks among neonates are rare. In the present study, rotavirus, calicivirus (noroviruses GI 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 HAstV positive by RT-PCR and corroborated by the ELISA data. These data suggest strongly that this outbreak was caused by HAstV.

HAstV-1 is the most prevalent serotype circulating globally, as well as the most prevalent HAstV 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 HAstV serotype 1. Furthermore, based on the phylogenetic analysis of the 348-bp region of the HAstV 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 (HAstV) serotype 1 without recombination between ORF1 and ORF2, and they indicate the importance of HAstV-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 HAstV can cause severe diarrhea among neonates.

The outbreak lasted more than 4 months. HAstV incidence peaked twice during the outbreak (Fig. 1), which suggests that HAstV 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 HAstV. 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 HAstV infection. A lack of environmental samples from the neonatal care room meant that direct evidence of the relationship of infection and environmental persistence of HAstV was not obtained.

This study is to our knowledge the first to link a particular HAstV serotype (HAstV-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 HAstV in outbreaks of gastroenteritis and provide more information on the extent of HAstV 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.

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None of us has a conflict of interest.

**REFERENCES**

