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 (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 ward 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 24 September 2008) developed diarrhea. 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 HAstV-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). HAstV detection rates in male and female subjects were both 70.0% (21 of 30 and 7 of 10, respectively). HAstV 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.

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-

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

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.
n 61 subjects, 15 (24.6%) had underlying conditions, broken
7.95 per day (95% CI, 7.38 to 8.52; range, 4 to 11/day). Of the
range, 1 to 10 days). The mean frequency of loose stools was
38.4°C). No vomiting was noted in any subject. The mean
duration and of less severity than that caused by other enteric
-duration, and of less severity than that caused by other enteric

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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, respec-
tively. The sequences of reference strains were obtained from
GenBank for comparison with sequences obtained in this study.

HAstV persisted in the environment during the outbreak and in
this way infected previously healthy subjects. Indeed, previ-
ous studies have reported that astrovirus can persist for ap-
proximately 2 months on contaminated surfaces (5). Persist-
ence 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 relation-
ship 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.

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 com-
parison of ORF1a and ORF1b/ORF2 nucleotide sequences
suggested that the Inner Mongolia strains were not recombi-
nant 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 vom-
iting 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, previ-
ous studies have reported that astrovirus can persist for ap-
proximately 2 months on contaminated surfaces (5). Persist-
ence of astrovirus on contaminated surfaces within the
neonatal care room may explain the lengthy duration of the
outbreak.

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), intraventricular 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 spec-
imens 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, respec-
tively. Of the 28 positive subjects, 7 had underlying conditions,
and there were 3 in the 12 negative subjects. The mean 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 fre-
quently 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, 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 HAstV positive by RT-PCR and corroborated
by the ELISA data. These data suggest strongly that this out-
break was caused by HAstV.

Astroviral diarrhea has been regarded as being shorter in
duration and of less severity than that caused by other enteric

multiply the 289-bp ORF1a and 1,260-bp ORF1b/ORF2 regions
using primers Mon340/Mon348 and Mon344/Mon270, respect-
ively. 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.

Clustered into the same branch of the HAstV-1 strain.

Clustered into the same branch of the HAstV-1 strain.