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Group B rotaviruses similar to strain CAL-1, have been circulating in Western India since 1993

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SUMMARY

Generally, group A rotaviruses are the most common cause of paediatric diarrhoea. However, group B rotavirus, adult diarrhoea rotavirus (ADRV), was found to be involved in epidemics of severe gastroenteritis in several areas of China during 1982–1983 and had resulted in more than one million cases among adults as well as older children. Human group B rotavirus has been rarely reported outside China, but has been detected first from five adults with diarrhoea in Kolkata, India during 1997–1998 (strain CAL-1). During epidemiological studies at the National Institute of Virology (NIV) on hospitalized diarrhoea patients at Pune, India, faecal specimens from patients of >5 years age, which were negative for group A rotavirus by ELISA were tested by polyacrylamide gel electrophoresis (PAGE). We detected rotavirus RNA migration patterns similar to that of group B rotavirus in three faecal specimens from adults, two from the specimens collected in 1993 and one in 1998 from sporadic diarrhoea cases. RT–PCR was carried out using primers derived from gene 8 which codes for the NS2 protein, followed by nested PCR, which confirmed the presence of group B rotavirus in all three specimens. The sequences of the PCR products of NIV specimens were compared with that of CAL-1, ADRV and IDIR (infectious diarrhoea of infant rat) belonging to group B rotaviruses. The sequence analysis of the PCR products showed the highest identity with CAL-1, which was reported from Kolkata, India during 1997–1998. The finding suggests that human group B rotaviruses have been circulating in Pune, India, since 1993. This emerging virus may lead to more severe disease among adults in India. There is a need for surveillance of group B rotavirus infections, especially in adult diarrhoea cases and seroepidemiological studies on group B rotavirus are required among humans and animals of Western Maharashtra, India.

INTRODUCTION

Group B rotaviruses have been identified in humans, pigs, sheep, cattle and rats. Adult diarrhoea rotavirus (ADRV) belonging to group B rotavirus was involved in epidemics of severe gastroenteritis in several areas of China during the 1980s [1]. In these epidemics, all age groups, particularly adults were affected. Outside China, there are only two reports of group B rotaviruses in humans, both from Maryland, USA [2, 3]. Krishnan et al. [4] reported five cases of severe adult diarrhoea due to infection with group B rotavirus, confirmed by RT–PCR from Kolkata, India during 1997–1998. Sen et al. [5] showed that the CAL-1 strain is the prototype or wild-type strain and suggested that this may be a commonly occurring human pathogen that has so far remained undetected.

The genome of group B rotavirus comprises 11 segments of double-stranded (ds) RNA like those of

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group A rotaviruses. However, the RNA migration patterns of group B rotaviruses lack a triplet of segments as seen in the 7–8–9 region of group A rotavirus RNA migration pattern and the RNA migration pattern of group B rotavirus from different species is not uniform.

We have previously reported the prevalence of group A rotavirus in children less than 5 years old, hospitalized with diarrhoea during a 4-year period from July 1992 to June 1996 [6]. During the same period, 1322 faecal specimens were collected from patients more than 5 years old, hospitalized with diarrhoea, the mean age being 36·85 years. The specimens were examined by ELISA for group A rotavirus [7] and also by employing a group A-specific monoclonal antibody (obtained from Dr S. Urasawa, Japan). Eighty-five (6·4%) of these 1322 specimens, were positive for group A rotavirus by ELISA and the remaining 1237 were negative. A total of 290 specimens out of 1237 were from participants $\geqslant$18 years old collected throughout 1993. Of these 275 faecal specimens were tested by polyacrylamide gel electrophoresis (PAGE) in order to detect non-group A rotavirus, if any.

METHODS

Briefly, RNA was extracted from a 20% stool suspension in PBS with 0·01 M $CaCl_2$ using phenol and precipitated with ethanol. The RNA pattern was studied on 10% (w/v) PAGE [8]. As we could detect an RNA migration pattern similar to that for group B rotavirus in specimens collected during June and July 1993 retrospectively, 101 faecal specimens were also collected during June–July 1998 from hospitalized adults with diarrhoea who were more than 18 years old to examine for the presence of group B rotavirus.

Specimens showing an RNA pattern similar to that of group B rotavirus were confirmed by RT–PCR as described by Gouvea et al. [9] using primers from the sequence of gene 8, which codes for the NS2 protein. The sequence (5′ to 3′) of forward primer B1 (position 18–39) was 'CTATTCAGTGTGTCGTGAGAGG', reverse primer B3 (position 430–51) was 'CGAAGC GGGCTAGCTTGTCTGC' and reverse primer B4 (position 486–506) was 'CGTGGCTTTGGAAAA TTCTTG'. Primers B1 and B4 were used for one-step RT–PCR and B1 and B3 for the following nested PCR. The expected PCR product size of nested PCR was 433 bp. Nucleotide sequencing of the nested PCR product was performed using auto sequencer

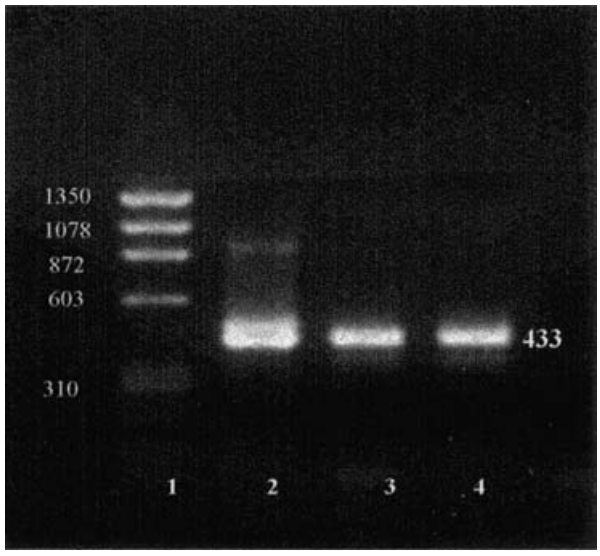

Fig. 1. RT–PCR of NIV specimens (product size, 433 bp). Lane 1, ladder (MW bp): 1350, 1078, 872, 603, 310, 271, 234, 134, 118, 72; lane 2, NIV specimen no. 935436; lane 3, NIV specimen no. 984679; lane 4, NIV specimen no. 935893.

ABI Prism® 310 Genetic Analyzer from Applied Biosystems (Foster City, CA, USA).

RESULTS

A total of 275 specimens out of 1322, negative by group A rotavirus ELISA were tested by PAGE. Two specimens collected from adults, one in June and another in July 1993 showed a rotavirus RNA migration pattern, typical of group B rotavirus although the patients came from different localities. Of the 101 faecal specimens, collected during June and July 1998, one specimen showed an RNA migration pattern typical of group B rotavirus. We detected a group B rotavirus-like RNA migration pattern in three specimens, details of which are presented in the Table.

All three specimens were positive by RT–PCR (Fig. 1). Nucleotide sequences of the nested product showed a high level of identity with the nucleotide (nt) sequence of gene 8 of the CAL-1 strain, which was detected during the year 1997–1998 in Kolkata, India. Thus, all three strains were confirmed as group B rotavirus. The specimen collected in 1998, showed a higher level of identity to CAL-1 than the specimens collected in 1993. The CAL-1 strain appears to be more closely related to ADRV than IDIR (Figs 2 and 3). Amino-acid sequences of National Institute of Virology (NIV) strains and

Table. *Information regarding adult patients infected with group B rotaviruses, admitted to the Infectious Diseases Hospital, Pune, India*

NIV specimen no.	Date of hospitalization	Age (years)	Sex	Location	Distance from Pune station
935436	2 June 1993	19	Male	Hadapsar	11 km (east)
935893	13 July 1993	25	Female	Bhor	60 km (south)
984679	1 June 1998	27	Male	Kasba Peth	3 km (south-west)

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CAL1-AF205850 CTGAAGATGG ATATATGCCA TCAGACAGAG AATGTGTTGC ATTGGATAGA  [125]
NIV-935436    .......... .......... .......... .......... ..........  [ 75]
NIV-935893    .......... .......... .......... .......... ..........  [ 75]
NIV-984679    .......... .......... .......... .......... ..........  [ 75]
ADRV-M91437   .......... .......... ..G..T.... .G...A.... ......C...  [145]
IDIR-U03558   ....T..... .......... .......... .........T .C.A......  [145]

CAL1-AF205850 TATTTATCCA AAGAGCAGAA GGAACTAAGA GAAACTTTTA AGGATGGAAA  [175]
NIV-935436    .......... .......... .......... .......... ..........  [125]
NIV-935893    .......... .......... .......... .......... ..........  [125]
NIV-984679    .......... .......... .........G .......... ..........  [125]
ADRV-M91437   .......... .......... A.....G... .....G.... ..........  [195]
IDIR-U03558   ...C.G.... ....A..... ....T.G... ...GAG.A.. .A..C.CT..  [195]

CAL1-AF205850 AAATGATAGA TCAGCTTTAA GAATTAAAAT GTTCTTATCT CCTTCACCTT  [225]
NIV-935436    .......... G.....C... .......... ...T...... ..C.......  [175]
NIV-935893    .......... G.....C... .......... ...T...... ..C.......  [175]
NIV-984679    .......... .......... .......... ...T...... ..........  [175]
ADRV-M91437   ...C...... G.....C... .......... ...T....GC ..........  [245]
IDIR-U03558   .......... G.T.G.C.GC ..G.C..... ...T..G..C ..A.....G.  [245]

CAL1-AF205850 CCAGACGATT CACTCAACAT GGAGTTGTTC CAATGAGAGA AATAAAAACA  [275]
NIV-935436    .......... .......... .....C.... .......... ..........  [225]
NIV-935893    .......... .......... .....C.... .......... ..........  [225]
NIV-984679    .......... .......... .......... .......... ..........  [225]
ADRV-M91437   .......... .......... .....C.... .......... ..........  [295]
IDIR-U03558   .A..GA.... T......... .........G. .......G.. G..T..G..G  [295]

CAL1-AF205850 AATACGGATA TACCAAGTAC ACTATGGACT CTTGTGACTG ATTGGTTACT  [325]
NIV-935436    .......... .......... .......... .......... ..........  [275]
NIV-935893    .......... .......... .......... .......... ..........  [275]
NIV-984679    .......... .......... .......... .......... ..........  [275]
ADRV-M91437   .......... .G........ ...G...... .......... .......G..  [345]
IDIR-U03558   .G.G.T.... ........T. .T..CA..G. A..A.C..A. .....C.GT.  [345]

CAL1-AF205850 AAATTTACTT CAAGATGAAG AAAATCAGGA AATGTTTGAA GATTTTATTA  [375]
NIV-935436    .......... .......... .......... .......... ..........  [325]
NIV-935893    .......... .......... .......... .......... ..........  [325]
NIV-984679    .......... .......... .......... .......... ..........  [325]
ADRV-M91437   ......G... ..G....... .......... .......... .....C....  [395]
IDIR-U03558   G...C..... ..G..C.... .G..C..... .......... ..C..C..A.  [395]

CAL1-AF205850 GTTCCAAATT TCCGGATGTT TTAGCTTCGG CAGACAAGC [415]
NIV-935436    .......... .......... .......... ......... [365]
NIV-935893    .......... .......... .......... ......... [365]
NIV-984679    ....A..... .......... .......... ......... [365]
ADRV-M91437   ....T..... .......... .......... ......... [435]
IDIR-U03558   A...T..... C..A..CA.. C.C..G..A. .T......T [435]
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Fig. 2. Multiple sequence alignment of NIV specimens and subject sequence CAL-1 for segment 8 (nt no. 76-414), using Clustal 1.8X.

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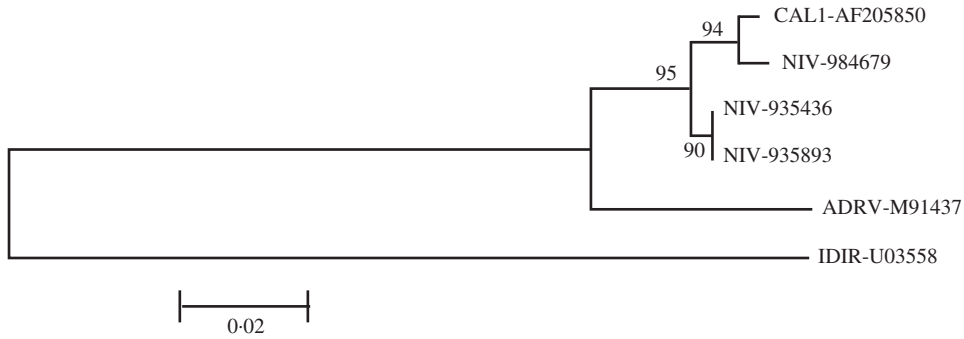

Fig. 3. Phylogenetic analysis of NIV-935436, NIV-935893, NIV-984679, CAL-1 AF205850, ADRV (adult diarrhoea rotavirus) and IDIR (infectious diarrhoea of infant rat) using molecular evolutionary genetic analyser (MEGA) version 2.1.

CAL-1 were studied. There was a change in one amino acid (serine to alanine) at position 51 in both the NIV 93 strains when compared with the CAL-1 and NIV 98 strains.

DISCUSSION

The detection of group B rotavirus is important from the public health viewpoint because group B rotaviruses cause severe diarrhoea mainly affecting adults. The intriguing point is that group B rotavirus only occurs sporadically in Pune, India compared to the explosive outbreaks reported from China during the 1980s. It could be detected in a very small number of cases and apparently is not spreading to cause large-scale outbreaks in adults. Our results show that the virus is circulating at a low level. Group B rotavirus infection among children (5·4%) and adults (2·9%) has been also reported recently from Bangladesh [10].

It has been reported that degradation of group B rotavirus particles is frequently seen in newly collected stool samples [1]. This could be one of the reasons why group B virus has not given rise to major outbreaks more recently and is implicated as a causative agent only in sporadic cases so far. Humans are probably an important reservoir for ADRV but transmission through person-to-person contact may be very low. However, the virus may alter genetically and cause outbreaks.

Recently, during 2000, an epidemic of diarrhoea occurred in Daman Union territory, situated on the western coast of India approximately 300 km from Pune. We detected three faecal specimens positive to group B virus from the same location. All three strains were similar to NIV 98 and CAL-1 strain (results not shown).

Although outbreaks of group B rotavirus have occurred in the mainland of China, infection with ADRV- or ADRV-related viruses was widespread. Antibodies were detected in approximately 10% of healthy adults in China [11] and were also detected at low levels in other countries, where the disease has not yet been reported (e.g. Australia, United States, Kenya, Thailand [12], Hong Kong, Nepal, South Korea [13] and United Kingdom [14]). However, it is quite possible that sporadic cases are occurring at a low level. Aetiological agents of a large percentage of diarrhoea cases among adults normally remain unknown.

The group B rotavirus antibody titres were four-fold higher in the gamma globulin (GG) pools collected in 1983 from China, just after the epidemic due to ADRV, than from the GG pools prepared approximately 5–6 years prior to 1982, but not in the GG collected before 1982 from American reference pools [15]. This suggests that ADRV was present in China prior to 1982 and spread there, and possibly to other countries such as India. This could be the reason for the similarity between the sequences of ADRV and Indian group B rotaviruses.

Antibodies to ADRV have been detected most frequently in home rats and pigs in different locations in China [16]. Thus, domestic animals may be the natural reservoir for ADRV- and ADRV-like viruses. Unfortunately, there are no seroepidemiological studies of group B rotavirus among animals in Western India; these need to be conducted, along with seroepidemiological studies in humans, to look for unapparent infections in humans.

Thus, it appears that group B rotavirus is circulating in India, causing sporadic but severe diarrhoea and warrants active surveillance among humans and animals.

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