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Human group C rotavirus in children with diarrhea in the Federal District, Brazil

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Abstract

Group C rotaviruses are fastidious in their *in vitro* cell culture requirements. Recent serosurveys indicate that antibody to group C rotavirus is present in 3-45% of the human population in certain geographic locations, suggesting that rotavirus group C infection is more prevalent than previously believed and that the low rate of detection of these agents is probably due to the lack of sensitive diagnostic assays. From March to December 1994, 406 fecal specimens were collected from children under five years of age who were outpatients at the emergency services of nine public hospitals in Brasília, Federal District, Brazil. In addition to the samples from children, one public outpatient unit requested virological investigation of a stool sample from an HIV-seropositive adult male with diarrhea of sudden onset. All samples were analyzed by enzyme immunoassay for group A rotavirus and adenovirus (EIARA) and by polyacrylamide gel electrophoresis (PAGE). One hundred and seven (26%) were positive for group A rotavirus. Four samples from children and the sample from the HIV-seropositive patient, although negative by EIARA, showed a group C rotavirus profile by PAGE and were positive for rotavirus by electron microscopy. Using specific VP6 and VP7 primers for group C rotavirus, a reverse transcriptase-polymerase chain reaction (RT-PCR) was performed and products were detected by agarose gel electrophoresis and ethidium bromide staining. These products were confirmed to be specific for group C rotavirus by using digoxigenin-oligonucleotide probes, Southern hybridization and chemiluminescent detection. The five positive group C rotavirus samples were detected in August (3 samples) and September (2 samples). To the best of our knowledge, this is the first report of group C rotavirus detected in the Federal District, Brazil and in an HIV-seropositive patient with acute gastroenteritis.

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- Diarrhea
- HIV
- RT-PCR
- Probe hybridization

Introduction

Rotaviruses are members of the *Reoviridae* family which possess a triple capsid layer of concentric icosahedral shells surrounding a genome containing 11 segments of double-stranded RNA (dsRNA). Initial studies using various serologic techniques have established that rotaviruses from diverse species share a common group antigen located on the inner capsid layer (VP6). However, antigenically distinct rotaviruses morphologically indistinguishable from conventional rotaviruses, but lacking the common group antigen, were identified in vertebrates (1,2). Based on these immunological characteristics, rotaviruses were classified into seven serogroups (A-G) with members of each group presenting a common antigen and a distinctive electrophoretic migration pattern of the 11 segments of dsRNA (1,2).

Group A rotavirus is the leading cause of severe diarrhea in young children and many species of animals throughout the world and is associated with 870,000 deaths/year in children under 5 years old in developing countries (3). Determination of prevalence and significance of non-group A rotavirus infections remains extremely difficult due to the lack of routine procedures. However, group B and C rotaviruses have been associated with human diarrhea in different countries of the world (4-13).

Recent serosurveys indicate that antibody to group C rotavirus is present in 3-45% of the human population in certain geographic locations, suggesting that rotavirus group C infection is more prevalent than previously thought and that low rates of detection of this group of viruses may be due to the lack of sensitive diagnostic assays (7,8,11,12,14). For example, in one study Jiang and coworkers (8) found that polyacrylamide gel electrophoresis (PAGE) or enzyme immunoassay (EIA) detected only 0 and 19%, respectively, of true group C rotavirus-positive fecal specimens.

Group C rotaviruses have been difficult to culture. At present, only relatively few group C rotaviruses have been successfully propagated in cell culture, including porcine Cowden, bovine Shintoku, and the human Ehime 9301 strains (15-17).

Diarrhea is a common manifestation among persons with the acquired immunodeficiency syndrome (AIDS). However, the etiology of these enteric diseases is not often determined. Viruses such as group A rotavirus, cytomegalovirus, adenovirus, astrovirus, human calicivirus, and picobirnavirus have been described in association with diarrhea in AIDS patients (18).

In Brazil, group C rotavirus was first described by Pereira et al. (19) and later by other groups (20-22) and also by Alfieri AA and Alfieri AF (unpublished results). We presently report the detection of group C rotavirus infecting children in the Federal District, Brazil, as well as, to the best of our knowledge, the first detection of this group of rotaviruses in one HIV-infected patient with acute diarrhea.

Material and Methods

Specimen collection

From March to December 1994, 406 fecal specimens were collected in the Emergency Service of nine public hospitals in the Federal District. The population comprised children under five years of age with a clinical history of diarrhea of up to 14 days duration. Individual epidemiological data were obtained for all patients. Although the children belonged to families in the low socioeconomic segments of the general population, the majority had good sanitary conditions, as indicated by the rates of public water (72%) and sewage (64%) facilities in their houses. In addition to the samples from children with diarrhea, who were the main subjects of this study, we received in August 1994 one fecal specimen from a 31-year-old

HIV-seropositive male with acute gastroenteritis living in the metropolitan area of Brasília.

The collected samples were either sent to the Public Health Laboratory of the Federal District within 2 h, or kept refrigerated at 4ºC until delivered to the laboratory within 48 h. Aliquots were sent to the Virology, Bacteriology, and Parasitology Laboratories of the Health Institute of the Federal District for routine analysis. The bacteriological investigation was carried out following the protocol described by Edwards and Ewing (23) while the parasitological investigation was performed following the protocols described by Neves (24) and Lima (25). For virus investigation, the fecal suspensions were prepared by diluting stool (10-20%) in phosphate-buffered saline, pH 7.2, followed by mixing and clarification at 3,000 *g* for 10 min at 4ºC. Extraction with an equal volume of 1,1,2 trichloro-1,2,2 trifluoro-ethane (Genetron, Duque de Caxias, RJ, Brazil) was used when necessary.

Enzyme immunoassay, polyacrylamide gel electrophoresis, and direct electron microscopy

All fecal suspensions were screened for group A rotavirus using an enzyme immunoassay for group A rotavirus and adenovirus (EIARA) (26), supplied by Bio-Manguinhos, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, following manufacturer recommendations.

Five hundred microliters of fecal suspensions were used for dsRNA extraction by the method of Boom and coworkers (27). The PAGE and silver staining methods have been previously described (28).

For direct electron microscopy (EM), 25 µl of fecal suspension was used on a 400-mesh copper-grid covered with Formvar and carbon. After 1 min, the excess was absorbed with filter paper and the specimen was stained with 2% uranyl acetate, pH 7.2, for 30 s and allowed to dry. The grids were examined with a Jeol 100 C electron microscope at 30,000X magnification.

Reverse transcriptase-polymerase chain reaction (RT-PCR), Southern hybridization, and chemiluminescent detection of group C rotavirus

RT-PCR for the group C rotavirus VP6 gene was carried out using the primers and amplification conditions described previously (8). For the VP7 gene the following set of primers was used: positive sense BMJ107 5' TGT TTG GAG ATG TGA TGA 3', nucleotides 546 to 563 and negative sense C7.10 5’ATT GCC CGA TGT CTG 3', nucleotides 999 to 1013. Southern hybridization and chemiluminescent detection with VP6 and VP7 probes were performed as previously described (30). The 5' end-labeled digoxigenin-oligonucleotide probes (dig-probe) for VP6 and VP7 genes were designed to be homologous to internal regions of the VP6 and VP7 gene PCR products. The VP6 probe was previously described and the VP7 probe was: HCP2 5’GGG CTG CAT TTG GTA GTG AC 3', nucleotides 614 to 633 (29).

Results

Of 407 fecal specimens analyzed by EIARA and PAGE, 107 (26%) were positive for group A rotavirus (Table 1). Five samples negative by EIARA for group A rotavirus showed a typical group C rotavirus profile by PAGE (Figure 1). These samples were analyzed by EM and a classical rotavirus morphology was observed (data not shown). Specific VP6 and VP7 primers for group C rotavirus were used in the RT-PCR and bands corresponding to both amplified segments (270 bp for VP6 and 467 bp for VP7) were observed after ethidium bromide staining (Figure 2). The RT-PCR products were confirmed by hybridization with 5’dig-labeled oligonucleotide probes (Figure 2). Four of

Table 1 - Monthly rates of rotavirus detection in children and in one HIV-seropositive adult patient in the Federal District, Brazil.

EIARA = Enzyme immunoassay for group A rotavirus and adenovirus. PAGE = Polyacrylamide gel electrophoresis. *One sample from an HIV-seropositive adult patient.

Month (N)	Group A rotavirus-positive samples by EIARA and/or PAGE	Group C rotavirus-positive samples by RT-PCR	Temperature (°C)	Precipitation (mm^3)
March (28)	5 (18%)	0	20.9	324.4
April (14)	1 (7.0%)	0	21.2	143.1
May (17)	3 (18%)	0	20.5	69.4
June (18)	9 (50%)	0	18.1	14.6
July (32)	22 (69%)	0	18.3	4.0
August (72)	40 (54%)	2* (2.7%)	20.3	0.0
September (43)	17 (37%)	3 (7.0%)	23.2	0.0
October (66)	5 (8.0%)	0	23.7	50.0
November (55)	1 (2.0%)	0	22.2	278.9
December (62)	4 (7.0%)	0	21.4	193.6
Total (407)	107 (26%)	5 (1%)		

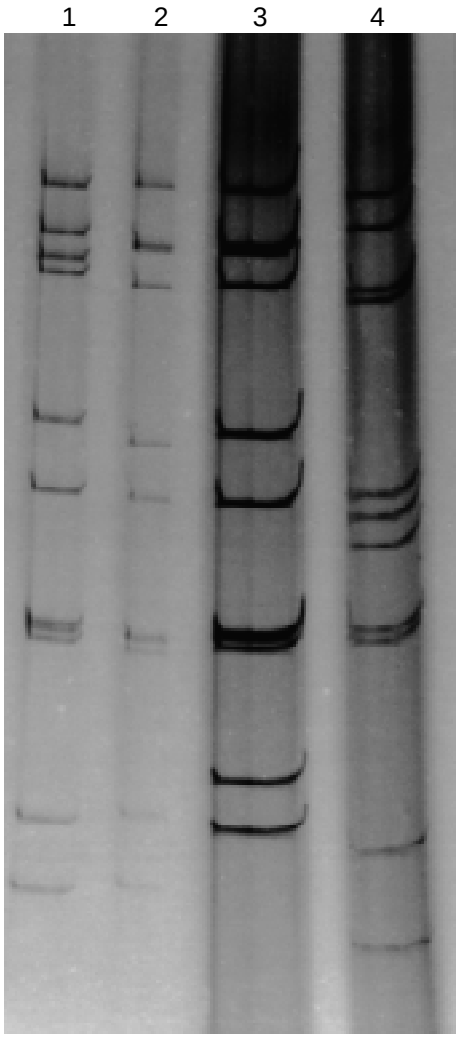

Figure 1 - Polyacrylamide gel electrophoresis of human rotavirus genomic dsRNA extracted from fecal specimens by the method of Boom et al. (27) and stained with silver. *Lanes 1 and 2*, group A rotavirus subgroup II; *lane 3*, group A rotavirus subgroup I; *lane 4*, group C rotavirus.

the five samples positive for group C rotavirus were from children and one from the HIV-seropositive adult patient.

Clinical records obtained for two of the children showed that they had received parenteral rehydration therapy and that they had fever (39°C), vomiting, and diarrhea lasting for 4 days. No other enteropathogen was detected by the methods routinely used in our institution for investigation of bacteria and parasites in patients with acute gastroenteritis. The adult HIV-seropositive patient had diarrhea for five days, vomiting, and fever (39°C). His absolute $CD4^+$ lymphocyte count was 252 cells/mm^3.

Discussion

This is the first study to identify group C rotavirus in fecal specimens in the Federal District, Brazil, after more than 12 years of rotavirus surveillance. In a previous study carried out in the Federal District from 1986 to 1990, we analyzed 607 fecal samples from children under five years of age by EIARA and PAGE, and detected a 20% rate of positive samples for group A rotavirus and no sample positive for group C rotavirus (31). In the current study group C rotavirus was detected in 4.5% of the rotavirus-positive samples between August and September, 1994. This period corresponds to the dry season, when the incidence of rotavirus A is highest (31; Table 1). The pediatric patients came from three distinct regions of the Federal District: two of them from urban areas of Ceilândia and Guará, two from Planaltina and the other from a rural area. Analysis of the social and sanitary conditions revealed that their homes were shared by an average of 10 people and that only one of them was connected to the public water and sewage network. Two homes had no public water or sewage and one had only public water. One of the families did not have domestic animals, three others had dogs, and the rural family raised pigs. Two children had signs of

severe dehydration and all samples were obtained within the first four days of symptoms.

Diarrhea occurs frequently among people with AIDS, but sometimes the cause remains unknown. In this study, we report one case of an HIV-seropositive patient with diarrhea associated with group C rotavirus, with no other enteropathogen being detected in his fecal specimen. Although this was just one case, we chose to report it here because, to the best of our knowledge, this is the first report of group C rotavirus in an HIV-positive patient. Further studies of the prevalence of group C rotavirus in HIV patients with diarrhea are warranted.

As shown in the present study, group A rotavirus can be detected in stools of up to 69% of children with diarrhea seen at emergency rooms, depending on the season. The frequency of diarrhea associated with group C rotavirus is low in comparison with group A infections, although group C rotaviruses have been detected in many different regions of the world (4-13). In Brazil, group C rotavirus was observed in Rio de Janeiro (Southeast region) (19), Belém (North region) (20), São Paulo (Southeast region) (21), Goiânia (Central-West region) (22), and Londrina, PR (South region) (Alfieri AA and Alfieri AF, unpublished results). The strains from Rio de Janeiro and Belém showed a closely similar electrophoretic profile, which was quite different from the one

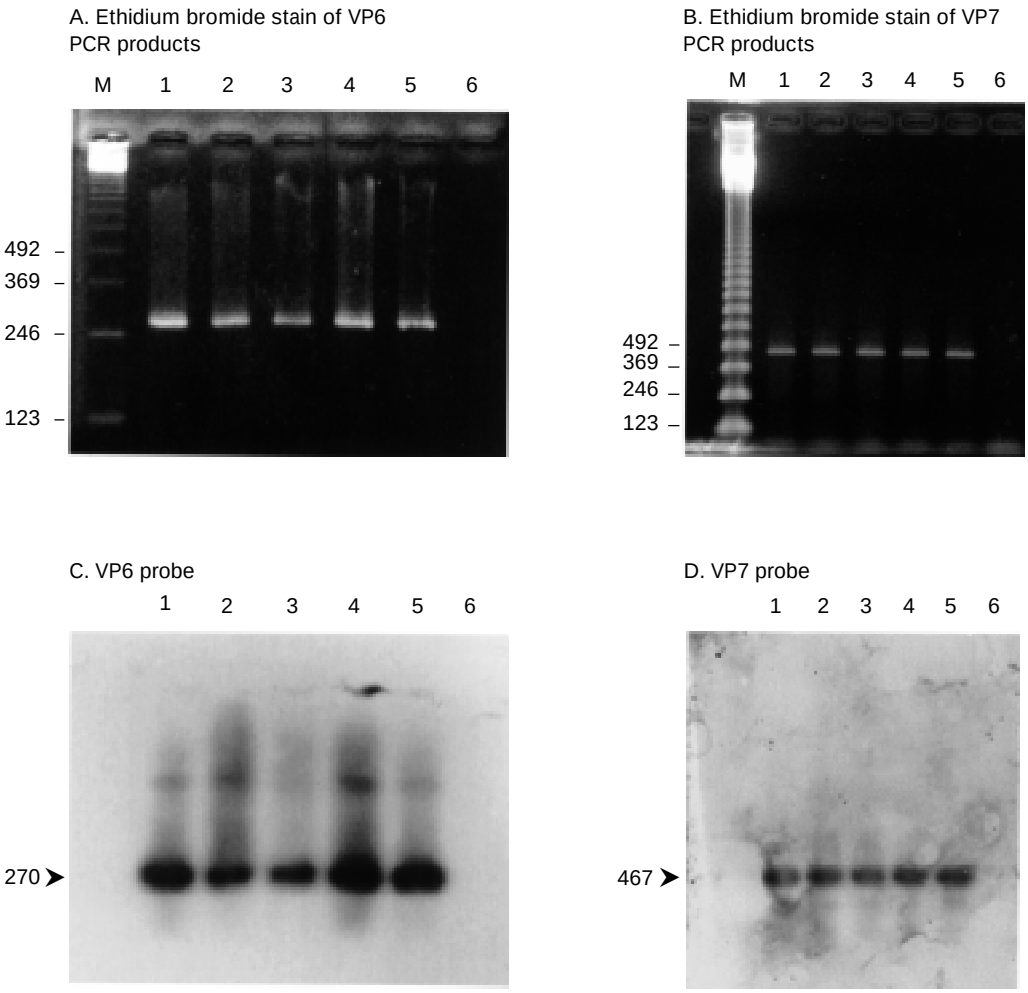

Figure 2 - Detection of group C rotavirus VP6 and VP7 RT-PCR products with a digoxigenin-labeled oligonucleotide probe. *A*, Ethidium bromide-stained 3% agarose gel showing the 270-bp VP6 RT-PCR products: M, 123-bp ladder molecular weight marker (GIBCO-Bethesda Research Laboratories, Inc., Gaithersburg, MD); *lanes* 1-5, Brazilian group C rotavirus strains; *lane* 6, negative control (water). *B*, Ethidium bromide-stained 3% agarose gel showing the 467-bp VP7 RT-PCR products: M, 123-bp ladder molecular weight marker (GIBCO-Bethesda Research Laboratories, Inc.); *lanes* 1-5, Brazilian group C rotavirus strains; *lane* 6, negative control (water). *C*, Southern blot hybridization and chemiluminescent detection with the VP6 probe. *D*, Southern blot hybridization and chemiluminescent detection with the VP7 probe.

observed for the strains from the present study. RNA segments 8 and 9 co-migrated in the strains from Rio de Janeiro and Belém, and did not show this pattern in the strains from the Federal District (data not shown).

Unfortunately, non-group A rotaviruses are not frequently investigated because of the lack of routine diagnostic methods. However, reports of group C rotavirus infections have increased in recent years. In Japan, group C rotavirus has been found in several areas and it is possible that in the forthcoming years these viruses might become widespread (13). Jiang et al. (8) described for the first time group C rotavirus circulating in the USA and showed that PAGE and EIA assays (12) had much lower sensitivity than RT-PCR to detect this group of rotavirus. Recently, Riepenhoff-Talty and colleagues (32) suggested a possible relationship between group C rotavirus and extrahepatic biliary atresia.

Since group C rotaviruses have been observed in different regions of Brazil, it seems important to increase the awareness of the rotavirus surveillance laboratories about these viruses, especially in samples from patients with diarrhea which are negative for more common group A rotaviruses.

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