An Outbreak of Group C Rotavirus Gastroenteritis Among Children Attending a Day-care Centre in Belém, Brazil

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ABSTRACT

In August 1993, an outbreak of group C rotavirus-associated gastroenteritis occurred among children attending a day-care centre in Belém, Brazil. Of the 64 children, 21 (33%) became ill. Group C rotavirus was identified in faecal specimens from 8 (38%) children with diarrhoea by electron microscopy (EM) and an enzyme immunoassay (EIA), using antibodies specific to the Cowden strain of porcine group C rotavirus. By polyacrylamide gel electrophoresis (PAGE), a pattern similar to that of group C rotavirus was observed in 5 (62.5%) of the 8 EM- and EIA-positive samples. These 5 faecal samples were confirmed to be positive for group C rotavirus by reverse transcriptase-polymerase chain reaction, using specific VP6 and VP7 primers. This is the first report of an outbreak of diarrhoea in North Brazil associated with group C rotavirus. These findings suggest that group C rotavirus may be an important aetiological agent of diarrhoea in this region, which requires further study.

Key words: Rotavirus; Diarrhoea, Infantile; Gastroenteritis; Disease Outbreaks; Day-care centres

INTRODUCTION

Rotaviruses are important causes of diarrhoeal illnesses among infants and young children worldwide (1,2). Rotaviruses belong to the family Reoviridae, and are classified into seven antigenically and genetically distinct serogroups (A-G) (3), of which group A, B, and C are known to infect both humans and animals (4). Group A rotaviruses are associated with high morbidity and mortality, particularly in the developing countries, leading to an estimated 800,000 deaths per year among children aged less than five years (5). Group B rotaviruses were initially detected in cows and pigs, but in 1984, these agents were also associated with large epidemics of severe diarrhoea among children and adults in China (6-9). Group C rotaviruses were first isolated from piglets with diarrhoea in the USA (10), and were subsequently identified by Bridger et al. (11), in 1986, to be human pathogens. Since then, workers in several countries have detected group C rotavirus in association with both sporadic diarrhoeal illness (12-16) and outbreaks of diarrhoea (17-20) which appear to have a global distribution (21). A recent report by Riepenhoff-
Talty et al. suggest that group C rotaviruses may be involved in the pathogenesis of extra-hepatic biliary atresia in infants (22).

It is likely that the incidence of group C rotavirus infection in humans is severely underestimated, because appropriate diagnostic methods, PAGE of RNA and electron microscopy, are not used in most clinical laboratories, and are rarely used for routine detection. In addition, more sensitive techniques, such as reverse transcriptase-polymerase chain reaction (RT-PCR) and enzyme immunoassays with group C rotavirus-specific reagents are available only in a few reference centres (12,15).

A recent serosurvey, carried out in Sweden, indicates that the prevalence of antibody to group C rotavirus ranges between 35% and 45%, depending on age (23). In the UK, a survey, involving 1,000 human serum samples, obtained from all age-groups and screened by an enzyme-linked immunosorbent assay (ELISA), yielded an overall seroprevalence of 43%, with the highest rate of 66% in the 71-75-year age-group (24). These results strongly suggest a high level of exposure of humans to group C rotaviruses throughout life.

In Brazil, group C rotaviruses were first identified by Pereira et al. (25) in a faecal specimen from a child with gastroenteritis in Rio de Janeiro, and have since been detected in other regions of the country, including São Paulo, Santa Catarina, Belém, Brasilia, and Valentin Gentil (26-30). The present report documents an outbreak of diarrhoea caused by group C rotavirus, affecting at least 38% of the children who attended a day-care centre (DCC) in Belém, Pará, Brazil.

MATERIALS AND METHODS

Patients and faecal specimens: During July 1993-June 1995, we conducted a regular surveillance of acute diarrhoea among children aged less than five years who attended a DCC in Belém, Pará. Children were visited twice a week to detect outbreaks of gastroenteritis. In addition, the DCC staff telephoned us whenever cases of diarrhoea occurred between visits. The DCC was attended by children from low-income families who were, in general, undernourished at admission.

On 18 August 1993, we were notified of an outbreak of diarrhoea affecting 21 (33%) of the 64 children aged 10 months to 3 years. This was the second outbreak of a series of nine which occurred in this DCC during the survey period. The clinical symptoms were, in general, mild, and diarrhoea resolved without leading to dehydration. Faecal specimens were collected from all children with diarrhoea and 4 from staff members who had no symptoms. These specimens were examined for bacterial and parasitic pathogens and stored at –20 ºC until processed for viral diagnosis.

Enzyme immunoassay (EIA): Stools were diluted to make a 10%-20% suspension in phosphate-buffered saline and centrifuged at 3,000xg for 15 minutes at 4°C. The supernatants were tested for the presence of group A rotavirus and adenovirus antigens, using an EIA (EIARA) provided by Bio-Manguinhos, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil (31). To detect group C rotavirus antigen, we used a specific EIA kit, as previously described (12,32). This consists of a sandwich assay in which a polyclonal hyperimmune gnotobiotic pig serum (U 340) to the group C Cowden strain acts as a capture antibody and a biotinylated hyperimmune gnotobiotic pig serum (U339) to the Cowden strain as a detector antibody. These samples were also tested for the presence of astroviruses, using an EIA kit prepared at the Centers for Disease Control and Prevention (CDC), as previously described (33).

Polyacrylamide gel electrophoresis: Deproteinized rotavirus RNA was electrophoresed in 5% polyacrylamide gel, using a discontinuous buffer system (34), and was stained with silver nitrate (35).

Direct electron microscopy (DEM): Stools were examined by DEM after negative staining, using the method reported by Barth (36). Grids were stained with 2% phosphotungstate solution (PH 7.2), and were examined in a Zeiss EM 900 electron microscope at 40,000X magnification.

Reverse transcriptase-polymerase chain reaction: Group C rotavirus dsRNA was extracted from stool suspensions, as described by Boom et al. (37). The RT-PCR was carried out, using group C rotavirus-specific primers for the VP6 (primers BMJ41 and BMJ42) and VP7 (primers BMJ107 and C7-10) genes, as described previously (28). The mixture was amplified through 40 cycles of denaturation (94 ºC for 1 minute), primer annealing (48 ºC for 1 minute), and DNA polymerization (72 ºC for 2 minutes). The final cycle was followed by an extension period at 72 ºC for 10 minutes. The PCR products were analyzed by electrophoresis on 3% agarose gel at 100V, and were stained with ethidium bromide.

Bacteriological and parasitological procedures: Samples were tested for the presence of bacteria and parasites, following the techniques described in the WHO Manual for laboratory investigations of acute enteric infections (38).
RESULTS

During this outbreak, 21 (33%) of the 64 children had diarrhoea. Faecal specimens, collected from 21 children with diarrhoea and from 4 staff members who had no symptoms, were negative by EIA for group A rotavirus, adenovirus, and astrovirus. When tested by EIA for group C rotavirus, 9 (36%) were positive, including one sample from an adult who did not have diarrhoea. PAGE of dsRNA from the 21 samples showed 5 (24%) with the characteristic pattern of group C rotavirus, i.e. segment 5, 6, and 7 displaying a typical triplet pattern (Fig. 1) (39). When the results for group C rotavirus, obtained by PAGE and EIA, were compared, more specimens were positive by EIA (36%, 9/25) than by PAGE (20%, 5/25), and all samples were positive by PAGE were confirmed by EIA (Table).

All 9 specimens positive for group C rotavirus by EIA were also tested by RT-PCR, using the group C rotavirus VP6- and VP7-specific primers. PCR products with sizes of 270 bp and 467 bp for VP6 and VP7, respectively, were noted in five (3 VP6/VP7 and 2 VP6) of the diarrhoeic specimens (Fig. 2). Typical rotavirus particles were observed by DEM in all EIA-, PAGE- and RT-PCR-positive samples.

Of the eight diarrhoeic cases tested positive for group C rotavirus, five were detected on 18 August 1993, when most faecal specimens (one per child) were obtained. Entamoeba histolytica was detected in two (10%) patients, one of them was also group C rotavirus-positive. Mixed infection, involving group C rotavirus and Salmonella group D, was also detected in one child with diarrhoea. All the specimens from adults, including the group C rotavirus-positive one, were collected on 20 August.

DISCUSSION

This is the first report of an outbreak of diarrhoea associated with group C rotavirus in North Brazil. We had previously detected group C rotavirus in Belém in two children with sporadic episodes of diarrhoea (26). The detection of group C rotavirus in this outbreak was possible, because we were conducting a 2-year survey of diarrhoeal episodes in this DCC to assess the aetiologic roles of different viral enteropathogens. During this period, two of the nine outbreaks of diarrhoea recorded were associated with group A rotaviruses.
Outbreaks of group C rotavirus-associated gastroenteritis have been described throughout the world (15, 17-20). Most have involved either children aged one to five years or adults. In this outbreak, most children with group C rotavirus-associated diarrhoea in the DCC were aged about two years. Different age-groups were recorded in two outbreaks occurring in schools: one in London (England) (18) and the other one in Fukui (Japan) (19). Most patients were aged 4 to 12 years. However, group C rotavirus can be associated with gastroenteritis in infants as reported by Jiang et al. (12) in a 5-year survey conducted in the United States, which demonstrated, for the first time, the presence of group C rotavirus among hospitalized diarrhoeal children aged less than one year.

The detection of group C rotavirus in an asymptomatic member of the staff indicates possible transmission of this virus to adults, as reported elsewhere and the possible role of immunity with age (15, 20). Souza et al. (30) have reported that, during the same period as this outbreak in Belém, an outbreak of group C rotavirus-associated gastroenteritis occurred in Valentin Gentil, a small town of São Paulo State, affecting both adults and children.

The RNA profiles, identified during the outbreak, were identical to those noted in our previous study in Belém (26), and to that from the outbreak in Valentin Gentil, Southeast Brazil. This electropherotype similarity suggests a low degree of genetic variability for group C rotavirus strains in our region. This is in contrast to the findings of a study conducted by Ishimaru et al. (40), involving hospitalized children with acute gastroenteritis in Matsuyama, Japan, where two distinct RNA patterns were identified in different years (1985/1986 and 1988). In addition, both patterns, observed in the Japanese study, were different from those obtained in Belém, suggesting that group C rotavirus strains may differ in their genetic composition by region.

The first group C rotavirus isolate in Belém was analyzed by Cooke et al. (41) who sequenced PCR products from the VP6 gene. They observed that the Belém VP6 gene shares 98% nucleotide homology with that of the human group C/Bristol VP6 gene and 83% nucleotide homology with the corrected porcine group C/Cowden sequence. Subsequently, Grice et al. (42) compared the complete
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The nucleotide sequence of the Belém VP7 gene to the corresponding gene of the Bristol and Preston strains isolated in the UK, and showed that these genes were identical in size (1,063 bp) and nearly identical in sequence (97.8-99.8%). Recently, Jiang et al. (43) analyzed the VP7 gene from 14 human group C strains isolated from the USA and from four other continents, and found that these genes were highly conserved in predicted primary and secondary structures. Sequencing studies are planned with group C rotavirus strains obtained to date in Belém to assess the degree of nucleotide homology between them.

Two diagnostic techniques—the EIA specific for group C rotavirus and the PAGE—were compared in this study. ELISA was found to be more sensitive than PAGE. All EIA- and PAGE-positive samples were further tested by RT-PCR, using a pair of group C-specific primers, and five were confirmed. It is likely that more positive specimens might have been detected by RT-PCR, if all specimens, both ELISA-positive and ELISA-negative, had been examined, including additional (not used in the present study) internal primers (nested PCR).

In the present study, the clinical symptoms of diarrhoea due to group C rotavirus were milder than those reported for other two local outbreaks caused by group A rotavirus. Matsumoto et al. (19) also reported that group C infections were associated with mild clinical symptoms, characterized mainly by abdominal pain and vomiting in a large outbreak occurred in seven elementary schools in Fukui city, Japan.

We do not know the source of the group C strains, but interspecies transmission has been suggested previously (4). To establish this linkage, we plan to study group C rotavirus strains from pigs living in close contact with these children and see if the sequences of the VP6 and VP7 genes are closely related to those found in strains from our children.

Further studies are needed to understand the aetiology of group C rotavirus in diarrhoea among children and the epidemiology of group C rotavirus-associated infections in humans. The techniques of EM, EIA, and RT-PCR should be considered for routine use in the detection of group C rotavirus from faecal specimens of children or adults with diarrhoea.

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