

DETECTION OF HUMAN GROUP C ROTAVIRUS IN GHANAIAI CHILDREN

*G. E. ARMAH, E. K. ESSEL, R. H. ASMAH, F. R. ANTO¹, F. N. BINKA¹
Noguchi Memorial Institute for Medical Research, University of Ghana, P. O. Box LG 581,
Legon-Ghana. ¹Navrongo Health Research Centre, Ministry of Health,
P. O. Box 114, Navrongo-Ghana.

SUMMARY

Stool samples from 330 infants and young children aged between 1 and 24 months with diarrhoea were examined for the presence of rotaviruses by electron microscopy and ELISA. Double stranded RNA was obtained from the samples and their electropherotypes determined by polyacrylamide gel electrophoresis. Two specimens, which reacted negatively to group A rotavirus by ELISA but were positive by electron microscopy and polyacrylamide gel electrophoresis had an electropherotype pattern typical of human group C rotaviruses. This is the first report of the association of group C rotaviruses with diarrhoea illness in Ghana and extends the global distribution of group C viruses. The study further justifies the need for a continual surveillance of circulating rotavirus subgroups and subtypes especially in Africa where future rotavirus vaccines may be introduced to reduce/minimize severity of diarrhoea in children.

Keywords: Rotavirus, Group C, Ghana

INTRODUCTION

Rotaviruses are important viral agent responsible for gastroenteritis in infants worldwide and the single most important cause of nosocomially acquired, severe and dehydrating diarrhoea¹. Rotaviruses, members of the Reoviridae family, have also been cited as responsible for acute viral diarrhoea in many young animal species². Infants in both developed and developing world, regardless of the level of sanitation, are infected with the virus by the age of 24 months. These viruses are associated with approximately one million deaths per year worldwide in children under two years³.

The rotavirus genome consists of 11 double stranded RNA genes that code for both structural and non-structural proteins. Rotavirus is a triple-

layered particle which consists of a core encapsulating the RNA genome; an inner capsid which bears the group and subgroup antigen (VP6); and an outer capsid which has the viral neutralization antigens (VP4 and VP7). Based on these distinct antigenic and genetic properties, rotaviruses are classified into 7 groups (A – G). Group-A rotaviruses and at least two other groups B and C, are known to infect humans⁴⁻⁶. Group C rotaviruses, isolated in humans in many countries⁷⁻¹¹ are reportedly associated with extrahepatic biliary arthesia, a frequent form of ductal cholestasis that is a common diagnosis leading to liver transplantation in children. Group C rotaviruses have been also associated with outbreaks of diarrhoea and fatality in family outbreaks^{12,13}. Other studies indicate that antibody prevalence and incidence of group C rotavirus infection are highest in children ≥ 4 years old in contrast with group A rotavirus infection which is most common amongst children < 3 years of age^{14,15}. Although direct observation of viruses in faecal samples by electron microscopy can be employed to diagnose rotavirus infection, electron microscopy is a rather insensitive method and does not distinguish between rotavirus groups. Until the recent advent of reverse transcriptase polymerase chain reaction (RT-PCR) with group C specific primers, detection of non-A rotaviruses has been by direct electropherotyping. This method, however, required the presence of ≥ 10 ng of viral RNA for visualisation and the interpretation of the electropherotype profile was difficult and usually needed a confirmatory assay.

The introduction of newly licensed group A rotavirus vaccines into the Expanded Programme for Immunization (EPI) and subsequent development of immunity to group A rotaviruses, may bring to ascendancy the less competitive group C and B rotavirus in human populations. Furthermore group C rotaviruses cause more serious clinical sequelae than group B rotaviruses and their detection has far-

* Author for correspondence

reaching public health implications. There is therefore the need to determine the prevalence of circulating non A rotaviruses, especially group C rotaviruses, in the community. In Africa reports on group C rotavirus have been few and the only reports of group C rotavirus recovery has been from South Africa and Nigeria⁵⁻⁶. In this report we describe the identification of group C rotavirus from humans in Ghana, West Africa.

METHODS

Study area and sample collection

This study was carried out in the Kasena Nankana district of the Upper East region of Ghana. During the months of January to April 1999, 330 specimens were collected from children under two years as part of a rotavirus surveillance and rotashield vaccine immunogenicity studies in the district. The district has two main seasons, a short wet season from June to September and a long dry season for the rest of the year. The average annual rainfall is 950 mm and mean monthly temperature range from 20°C to 40°C. The population is rural except for a few (10 %) who live in the district capital Navrongo. The main occupation is farming and animal rearing. The people live in dispersed settlements in close contact with their farms and animals. Stool and rectal swab samples were obtained from children less than 2 years of age who sought medical attention at the only district hospital and health centres in the district and was stored at 4°C until tested.

Rotavirus Screening

Initially 10–20% faecal samples in phosphate buffered saline (PBS) pH 7.2 were screened for group A rotaviruses with the IDEIA Rotavirus kit (Dako Diagnostics Ltd, Cambridgeshire, UK) following the manufacturer's instructions. The IDEIA rotavirus kit utilises a polyclonal antibody in a solid-phase sandwich enzyme immunoassay to detect group specific antigen present in group A rotaviruses. In the case of swabs, they were soaked for 30 minutes at room temperature in 1 ml PBS and the suspensions used for the assays. The plates were read spectrophotometrically at 405 nm and each plate included a positive and negative control.

Electron Microscopy

All specimens that were negative for group A human rotaviruses ELISA were further examined by electron microscopy. The faecal suspension was clarified by centrifugation at 5000 rpm and 1 ml of clarified material layered on a sucrose cushion of 25% and centrifuged at 100,000g for 2 hr. The pellet was suspended gently in Tris buffer (pH 7.4)

and negatively stained with uranyl acetate (2%) on formvar coated copper grids. The grids were examined in a Hitachi H600 transmission electron microscope. Ten grid spaces were examined for each specimen and a specimen was ranked positive if virus was seen in any of the grid spaces and negative if no virus was seen.

RNA extraction and Polyacrylamide Gel Electrophoresis (PAGE)

All the stool samples were in addition further analyzed for human rotaviruses by PAGE to confirm the ELISA results as well as detect the potential presence of non A human rotaviruses. Approximately 20ul of extracted RNA were loaded and separated by electrophoresis on a 10% acrylamide gel using the discontinuous buffer system¹⁶. A 5% stacking gel was employed to enhance resolution of the RNA bands. Briefly, 15ul of each sample was loaded onto the gel and electrophoresed at 100V for 18 hours at room temperature. The gels were silver stained as described by Herring *et al*¹⁷ for visualization.

RESULTS

Of the 330 stool/swab specimens tested for group A rotaviruses by ELISA, 84 (25.5%) tested positive by group A ELISA. All those that were negative were tested for the presence of rotavirus by electron microscopy (Fig. 1). Sixty-seven (20.3 %) of the group A ELISA positive specimens yielded enough RNA that could be observed by PAGE as shown in Fig 2. They were all of the short electropherotype and showed patterns typical of group A rotaviruses (lane a). Two specimens (0.6%) which were negative by group A human rotavirus ELISA but positive by electron microscopy and PAGE were identified as group C rotaviruses from their electropherotype patterns (Fig.2: lane b). They showed the classic group C grouping of 4:3:2:2:2 dsRNA clusters. The group C rotaviruses were identified from stools of children aged 4 and 6 months. The clinical information of the two children with group C human rotaviruses were compared with those infected with group A rotaviruses. All children with group C rotavirus had diarrhoea for more than 3 days, vomiting for 3 days with at least 6 episodes /day and fever. The all showed symptoms of dehydration with a diarrhoea severity score based on that proposed by Ruuska and Versikari¹⁸ of 11.0 ± 0.2 compared with 9.5 ± 0.6 for those with group A human rotavirus. Electrophoretic patterns depicting mixed infections (extra RNA bands in the viral genome) were observed in two stool samples (Fig 2, lane c) Extra RNA bands were seen in the 1,2,3,4 and 5,6,7 clusters.

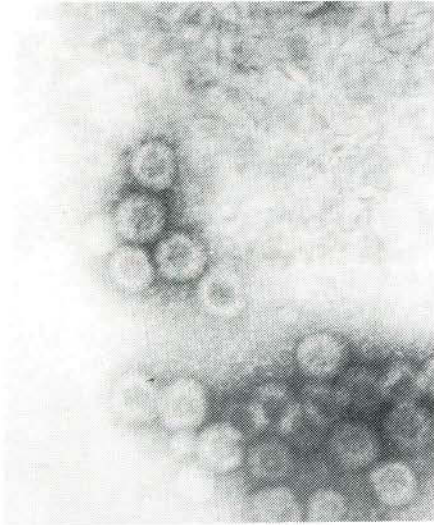


Figure 1 Rotavirus particles as observed by electron microscopy. Samples were stained with 2% uranyl acetate.

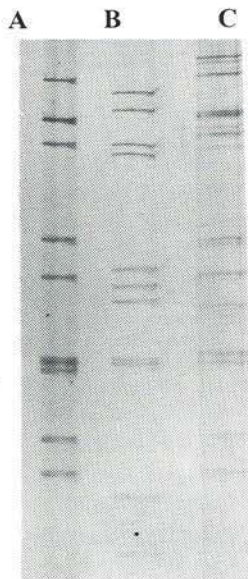


Figure 2 RNA electrophoretotype of group A (lane A), group C (lane B) rotavirus in Ghanaian stool samples. Lane C is mixed infections of group A human rotaviruses. Extra RNA bands can be seen in clusters 1234 and 567.

DISCUSSION AND CONCLUSIONS

Diarrhoea continues to be a major contributor to the high rate of childhood mortality in Africa. Of the 25 million children born each year in sub-Saharan Africa, about 4.3 million will not attain the age of six years and 20% of these deaths will be from diarrhoea. Rotaviruses have been identified as the single most important pathogen associated with severe life threatening forms of diarrhoea in children and

are responsible for 20-25% of these deaths. One of the approaches to reduce this high death rate and the burden of disease from rotavirus infection has been the development of an effective vaccine against this disease. These vaccines are however targeted only at group A rotaviruses but not at the non-group A rotaviruses that also infect. The sporadic nature of group C rotavirus infection has made its role in diarrhoea in children insignificant globally. However the fatality associated with group C rotavirus infection has been shown to be high in the few reported outbreaks¹³. The introduction of group A rotavirus vaccine in the community may provide a good opportunity for the upsurge of the less competitive group C virus. This fact coupled with the unassessed role of upsurge of concurrent infection with human immunodeficiency virus (HIV), enteric infections and malnutrition necessitates the need to put in place a surveillance system for circulating rotaviruses in the community. This preliminary study has shown that group C rotaviruses do circulate in Ghana. This present rate of isolation may be an underestimation due to the insensitive nature of the assays used in this study. Recent surveys in Brazil have however shown a high prevalence of serum antibodies to group C rotaviruses although detection of virus in stool specimens has been rather very low, further highlighting the underestimating of the burden of group C rotavirus worldwide. Group C rotaviruses have been reported to be more common in older people^{12,19}. The observation of mixed infections in the stool samples may be an indication of naturally occurring viral reassortment in the study area. A complete VP7 and VP4 (G and P typing) characterization of the mixed infections is presently in progress. We hope that the results from the G and P typing will help to address the issue of viral reassortment.

The inclusion of only group A rotavirus vaccine in the EPI programme in African countries may promote the proliferation of non-A rotaviruses. There is therefore the justifiable need to put in place a surveillance system for these viruses. It is hoped that the inclusion of much older children and the incorporation of much sensitive methods such as RT-PCR in studies currently in progress in Ghana will help in the determination of the prevalence rate and the disease burden in Ghanaians.

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