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# HUMAN GROUP C ROTAVIRUSES IDENTIFIED IN KENYA

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## ABSTRACT

***Background:*** **Rotavirus is associated with acute infantile gastro-enteritis in infants and young children worldwide. Rotavirus is associated with the high cases of morbidity and mortality and it is estimated that up to 650,000 deaths in young children occur annually in the less developed countries. Approximately a quarter of these deaths occur in African children yet minimal data exist on the circulating rotavirus strains in Africa.**
***Objective:*** **To characterise the circulating rotavirus strains in Kenya.**
***Design:*** **A prospective study to investigate rotavirus infection in infants and young children with acute diarrhoea in different areas of Kenya. Between September 2001 and February 2002, 119 faecal specimens were collected from children aged between 1 and 60 months with acute infantile gastro-enteritis.**
***Setting:*** **Faecal samples were collected from clinics around Nairobi and its suburbs including Karen, Ngong, Ongata Rongai**
***Subjects and Methods:*** **Faecal samples were collected from 119 infants and young children with diarrhoea and were analysed by commercial ELISA and polyacrylamide gel electrophoresis (SDS-PAGE) to identify possible non-group A rotaviruses. Extraction of any potential rrotavirus double-stranded RNA from faeces amd characterisation by SDS-PAGE showed the presence of human group C rotaviruses.**
***Results:*** **Examination of these samples with a commercial ELISA assay for the presence of group A rotavirus antigen showed that 13 specimens (11%) were positive. An analysis of all stool specimens was performed by polyacrylamide gel electrophoresis to identify possible non-group A rotaviruses. Extraction of any potential rotavirus double-stranded RNA from faeces and characterisation by SDS-PAGE showed the presence of human group C rotaviruses.**
***Conclusion:*** **This is the first report of group C rotaviruses in Kenya. Further studies are underway to continue the surveillance of rotavirus strains in Kenya; as this information will be useful in planning rotavirus vaccine trials in Africa.**

## INTRODUCTION

Rotaviruses are recognised as the important agents of acute infantile gastroenteritis in infants worldwide. Group A rotaviruses occur most commonly in infants and the young of many mammal species, including primates and are well characterised in the literature. Group A rotaviruses are associated with acute gastroenteritis in young children under three years of age worldwide(1). These viruses are the most important cause of severe dehydrating diarrhoeal illness in infants and are associated with approximately 875,000 deaths annually in this age group(1).

However, rotaviruses are classified into at least seven serogroups (A to G) on the basis of distinctive antigenic and genetic properties(2). Members of at least two of these other rotavirus serogroups (serogroups B and C) have been reported to occur in humans. Large outbreaks of group B rotavirus were reported in adults in China(3). Group C rotaviruses, on the other hand, have been associated with either sporadic diarrhoeal illness or limited outbreaks in various settings

Human group C rotaviruses have been described in several developed countries including Australia, USA, UK, Finland and Japan(4-8). In addition, the group C rotaviruses have been described in developing countries such as Latin America, India, China and Malaysia(9-12). Furthermore, there have only been limited reports of the recovery of group C rotaviruses in humans in Africa(13,14). In this report, we describe the first identification of group C rotaviruses from young infants in Kenya and in East Africa.

## MATERIALS AND METHODS

*Patients:* During the latter half of the year 2001 and the first two months of 2002, faecal specimens were collected from 119 infants and young children with diarrhoea in Kenya. This was part of an ongoing study to examine rotavirus infection and the molecular epidemiology of rotaviruses in Kenya. The specimens were collected from infants aged between one month and eight years of age, attending clinics at the University of Nairobi, College of Health Sciences Hospital and the surrounding health clinics. The specimens were examined for various viral agents including rotaviruses.

*Rotavirus detection:* Initially 10-20% faecal specimens were screened for the presence of the Group A rotavirus antigen using a commercially available ELISA test (Rotavirus IDEIA™ Dako UK). The test was performed as specified by the manufacturers and the results were read spectrophotometrically at 405nm.

*Polyacrylamide gel electrophoresis:* All faecal specimens were analysed by polyacrylamide gel electrophoresis (PAGE) to identify the potential presence of group B or C rotavirus double-stranded RNA (dsRNA) and to confirm the presence of group A rotaviruses. The methods have been described in detail elsewhere(15).

In brief the stool specimens were prepared as 10-20% faecal suspensions in distilled water, vortexed and then centrifuged at low speed to sediment any macroscopic debris. The suspension was initially mixed with an equal volume of 1M sodium acetate (pH 5) and 1% SDS to disrupt the proteinaceous material in the faecal specimen. This was followed by incubation with an equal volume of phenol/ chloroform incubation to de-proteinize the viral particles and release the dsRNA which was pelleted by centrifugation. The aqueous phase, which contains the dsRNA, was transferred to a clean Eppendorff tube and the dsRNA was precipitated in 3 volumes of absolute ethanol overnight at -20°C. After centrifugation at 12,000g, for 10min the pellet was re-suspended in Tris-EDTA buffer for the PAGE.

Standard electrophoresis was performed as described previously(15). In brief electrophoresis of the extracted dsRNA was performed in 10% polyacrylamide slab gels using the discontinuous buffer system described by Laemmli(16). A 5% stacking gel was used to enhance the resolution of the RNA segments. Approximately 20µl of each sample was loaded onto the gels and the electrophoresis was conducted at 100V for 16-18 hours at room temperature. The gels were stained by a modification of the silver staining method described by Herring *et al*(17), as described in detail by Steel and Alexander(15).

*VP6 Monoclonal antibody:* The VP6 subgroup-specificity of the rotavirus strains was determined by using the VP6 monoclonal antibodies developed by Greenberg *et al*(18). These monoclonal antibodies against subgroup I rotaviruses (clone 255/60) and subgroup II rotaviruses (clone 631/9) have been extensively used in studies worldwide. The methods for their use have been described in detail elsewhere(19). In brief microtitre plates were coated with the rabbit anti-rotavirus hyperimmune sera (sera 720) in a carbonate buffer and incubated with the rotavirus-positive stools overnight. The monoclonal antibodies were added afterwards and incubated for three hours at 37°C. A horseradish peroxidase conjugate was used for determination of the presence of the monoclonal antibodies (TMB Enzymatic Kit, Roche).

*Electron microscopy:* In addition, selected specimens were examined under the electron microscope. Transmission electron microscopy was performed with negative staining using 3% potassium phosphotungstate. Briefly, faecal samples were suspended in water and centrifuged for 30 min at 700g to eliminate macroscopic debris. Successive supernatants were centrifuged at 7000g for 30 Min and then at 48,000g for one hour. The final pellet was resuspended in a few drops of Tris buffer (pH 7.4) and negatively stained with 3% phosphotungstic acid (pH 6.4) on formvar-coated grids. These were examined in a Joel 100 CX transmission EM at a magnification of 40,000 times.

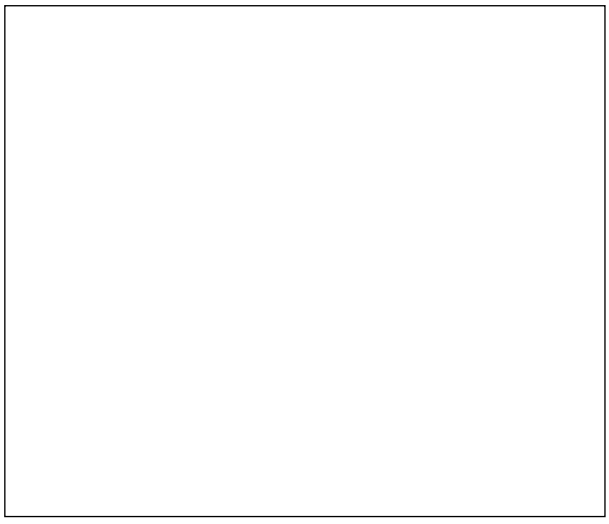
## RESULTS

In total, only 13(10.9%) of the specimens were positive by the group A ELISA. However, PAGE was performed on all 119 faecal specimens to investigate for the presence of non-group A rotaviruses. In addition to the PAGE RNA profiles that were seen with the group A ELISA positive rotavirus strains, an additional strain was observed with an RNA electropherotype by PAGE. It was interesting to observe that most of the Kenyan group A rotavirus strains revealed the short RNA profile.

The group C rotavirus was identified in the stool specimen of a young girl aged three and one-half years with acute diarrhoeal illness. Group A rotaviruses characteristically yield a standard configuration of the dsRNA segments after PAGE in a four, two, three, two pattern (Figure 1).

**Figure 1**

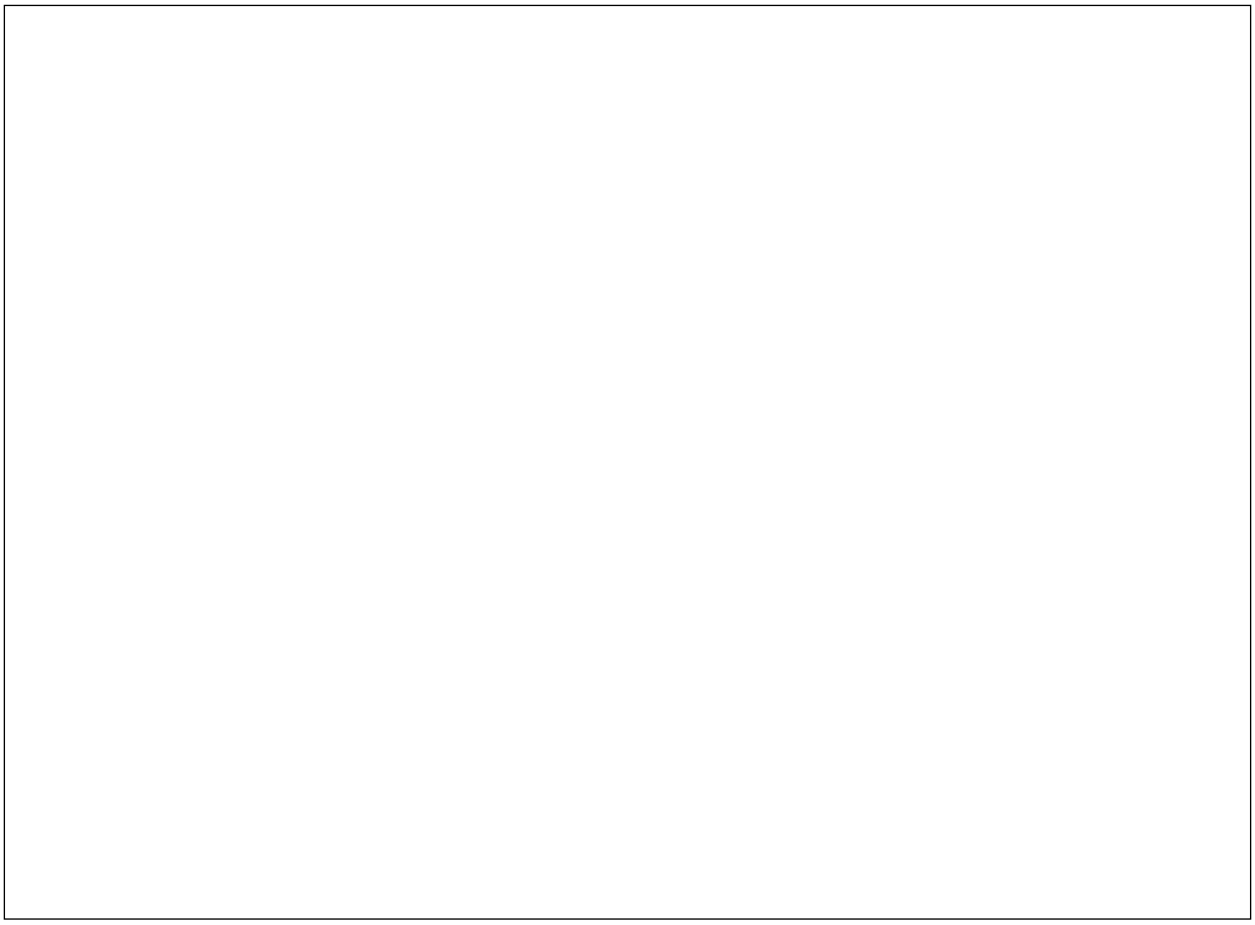
*Genomic dsRNA electropherotype of human group C rotavirus strain recovered in Kenya during 2002. The dsRNA segments are arranged in a 3,3,3,2 pattern that is indicative of group C rotaviruses*



The classical group C rotavirus RNA electropherotype can be seen where the dsRNA segments are grouped into a four, three, two, two configuration. Furthermore, typical rotavirus particles could be identified by standard negative staining electron microscopy in these stool specimens (Figure 2).

**Figure 2**

*Electron micrograph of group C rotavirus particles in the stool of a Kenyan child*



However, the group A-specific antigen was determined not to be present in this stool by both a commercial ELISA assay (Dako, UK) and by the monoclonal antibody to the VP6 group and subgroup antigens.

## DISCUSSION

In this study, we report the first identification of group C rotaviruses in East Africa in the diarrhoeal stools of a young child in Kenya. Furthermore, this study supports the recognition of the distribution of human group C rotaviruses in different regions in Africa(13,14).

Group C rotaviruses were first described in the diarrhoea stools of young children in the early 1980s, although the reports have always been limited in number(4-12). The identification of group C rotaviruses in Africa has also been limited with reports only from South Africa and Nigeria(13,14).

The role of group C rotavirus infection in humans has been considered to be insignificant in the global picture of diarrhoeal illness due to the apparent sporadic nature of occurrence. However, there has been a lack of sensitive techniques for the detection of these viruses with the development of more sensitive techniques and simpler ELISA-based assays(20), larger epidemiological surveys should be conducted. The human group C rotaviruses have been described to be more common in an older population than the group A rotaviruses(8,21).

The distribution and epidemiology of the group C human rotaviruses in Africa, using the newly developed recombinant reagents for ELISA-based assays, are important for several reasons. First, group C rotaviruses have been associated with both outbreaks of diarrhoea demonstrating their epidemic potential(21), and have been associated with fatality in a family-based outbreak demonstrating its potential virulence(22). Secondly, the distribution of group C rotaviruses has been shown to be more common than previously believed by a number of sero-epidemiological surveys for the presence of group C rotavirus antibody in humans.

Given the common sources of group C rotavirus strains in domesticated animals and environmental water, it would be expedient to consider conducting surveillance for these viruses using the newly developed techniques.

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