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Short Communication

Rotavirus Infection in Children with Diarrhea at Korle-Bu Teaching Hospital, Ghana

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SUMMARY: Human rotavirus infection was studied over a 13-month period (January 2004 to January 2005) in children <5 years of age admitted with severe diarrhea at the Korle-Bu Teaching Hospital in Accra, Ghana. During this period, 206 hospitalizations for diarrhea were recorded, with 34.0% (70/206) being positive for rotavirus infection. Infection occurred throughout the year, with peak rotavirus infection occurring during the month of March. Hospitalization associated with rotaviruses was most common in the 6–8 month age group. The case fatality rate of rotavirus infection was 2.9% (2/70) and occurred in children <12 months of age. Four rotavirus VP7 genotypes (G1, G2, G3, and G9) were detected. The predominant genotypes were G2 (22.9%), G1 (17.1%), G9 (17.1%) and G3 (12.9%). Mixed G types were also detected. The predominant VP4 genotypes (P types) were P[6] (38.6%), P[8] (21.4%), P[4] (4.3%) and P[9] (1.4%). The predominant rotavirus strains infecting children in Accra were G9P[6] (10.0%) and G1P[8] (8.6%). Strains with unusual genotypes such as G2P[8] and G(2/3)P[6] were also detected.

Rotavirus has been recognized as the single most important cause of severe diarrhea in children worldwide and an important public health problem, particularly, in developing countries (1–3). Prevention of rotavirus infection through vaccination appears to be the only effective option because rotavirus infections show a similar incidence in children throughout the world regardless of hygiene and development standards (3). Currently, there are 2 main rotavirus vaccines in use, including RotaTeq (Merck & Co., Whitehouse Station, NJ, USA) and Rotarix (GlaxoSmithKline, Rixensart, Belgium), and are based on VP7 and/or VP4 outer capsid proteins of the virus (4). The RotaTeq vaccine formulation includes G1–G4 genotypes and the human P[8] genotype, whereas the Rotarix vaccine includes G1P[8] which provides cross-protection against most other serotypes. The World Health Organization (WHO) recommends surveillance of the burden of rotavirus disease and the circulating rotavirus strains, before and after the inclusion of rotavirus vaccination in national expanded programs on immunization (5).

In Ghana, there are several surveillance reports on rotavirus (6–11). However, rotavirus surveillance appears to have been focused on the northern part of the country, where the climate contrasts sharply with that of southern Ghana. Rotavirus surveillance in Ghana provides evidence of the changing pattern of genotypes, which has important implications for vaccination (6). In this paper, we describe rotavirus infections and genotypes, including unusual gene variants, among children hospitalized with diarrhea at a tertiary hospital in southern Ghana prior to the introduction of the rotavirus vaccine in the country.

This study was conducted at Korle-Bu Teaching Hospital in Ghana from January 2004 to January 2005. All children aged <5 years admitted to the hospital with diarrhea on the day of the visit were included in this study. Diarrhea, in this study, was defined as the passage of more than 3 looser-than-normal stools in a 24-h period. Basic demographic and clinical information of the study participants was collected using a structured questionnaire. Fecal specimens were then collected from all eligible children into sterile containers and transported on ice to Noguchi Memorial Institute for Medical Research (NMIMR) in Accra, where they were stored at −20°C until rotavirus testing was performed. Follow-up data on the outcome of admission and the date of discharge were retrieved from hospital folders of all study participants and were entered into Microsoft Access (Microsoft, Redmond, WA, USA).

The protocol of the study was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School, and informed consent was obtained from the parents/guardians of the pediatric subjects enrolled in the study.

An enzyme-linked immunosorbent assay for rotavirus group A was performed using a commercially available DAKO Rotavirus ELISA kit (Rotavirus IDEIA; Dako Diagnostic, Cambridgeshire, UK) with a 10% suspension of fecal material in phosphate-buffered saline. Viral double-stranded ribonucleic acid (dsRNA) was extracted from the stool suspension by the Bender buffer

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extraction method (12,13). The cDNA of rotavirus VP7 and VP4 genes was produced in a reverse transcription-PCR (RT-PCR) reaction with VP7- and VP4-gene-specific primers (14–16). The PCR products were used as templates for a second round of amplification with a cocktail of genotype-specific primers that amplify variable regions of the VP7 and VP4 genes (14–16). The PCR products were electrophoresed on a 2.0% agarose gel containing ethidium bromide (Sigma-Aldrich, Steinheim, Germany) and visualized under UV illumination using a UV transilluminator.

Of the 206 stool samples collected, rotavirus was detected in 70 (34.0%) (Table 1). Infection occurred throughout the year, with peak rotavirus infection occurring in March (Fig. 1). Infection was most common in the 3–17 month age group (43.2%; 60/139) with a peak hospitalization of 52.6% in the 6–8 month age group (Table 1). In this study, more male children (34.5%; 40/116) had shed rotavirus than female children (33.3%; 30/90) (Table 1), however, this was statistically insignificant. Rotavirus infection was associated with a mean hospital stay of 4.8 days and a range of 1–46 days. Diarrhea mortality, in the study, was 3.9% (8/206), with a 2.9% (2/70) case fatality rate of rotavirus infection (data not shown). Four rotavirus VP7 genotypes (G1, G2, G3, and G9) were detected. The predominant genotypes were G2 (22.9%), G1 (17.1%), G9 (17.1%), and G3 (12.9%) (Table 2). Mixed G types were also common. Four VP4 types (P[6], P[8], P[4], and 1 unusual VP4 type, P[9]) were detected (Table 2). The predominant genotypes were P[6] (38.6%), P[8] (21.4%), P[4] (4.3%), and P[9] (1.4%) (Table 2). In this study, the predominant rotavirus strains infecting children in Accra were found to be G9P[6] (10.0%) and G1P[8] (8.6%). The following unusual genotypes and mixed infections were observed: G2P[8] (5.7%), G(2/3)P[6] (2.3%), G(3/4)P[4] (2.3%), and G(8/9)P[6] (2.3%).

Table 1. Age distribution of rotavirus infection

Age Group (Month)	Viral positivity		
	Man (%)	Woman (%)	Total (%)
0–2	2/17[1)] (11.8)	3/11 (27.3)	5/28 (17.9)
3–5	5/12 (41.7)	7/17 (41.2)	12/29 (41.4)
6–8	10/19 (52.6)	6/11 (54.5)	16/30 (53.3)
9–11	10/28 (35.7)	6/14 (42.9)	16/42 (38.1)
12–17	10/23 (43.5)	6/15 (40.0)	16/38 (42.1)
18–24	3/7 (42.9)	1/11 (9.1)	4/18 (22.2)
≥25	0/10 (0)	1/11 (9.1)	1/21 (4.8)
TOTAL (%)	40/116 (34.5)	30/90 (33.3)	70/206 (34.0)

[1)]: No. of positive samples/No. of samples tested.

The overall percentage of rotavirus infection of diarrhea cases detected in the present study (34.0%) is slightly higher than that previously observed in the Upper East Region of Ghana (25.5%) (7); it is also higher than that observed in Cameroon (21.4%) (17). It is, however, lower than the overall percentage of rotavirus infection observed in Navrongo in the Kassenna-Nankana District of the Upper East Region of Ghana (40.5%) (6). The present study has demonstrated that rotavirus infection occurred mostly in children below the age of 24 months. This conforms with previous studies in Ghana (8), West Indies (18), and other places (19,20). The sharp decline in rotavirus infection in children beyond the age of 17 months could be explained by the immunity build-up due to multiple exposures to rotavirus in the early part of life (21). The median age of rotavirus infection in the present study (6–8 months) is similar to that reported in previous studies (8,9). This is lower than the median age reported in the developed

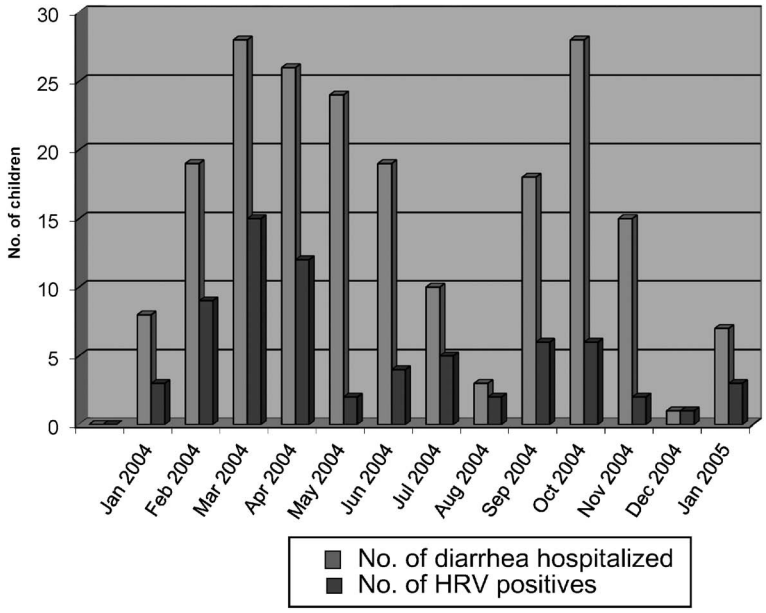

Fig. 1. Seasonality of human rotavirus (HRV) infection among the children studied over a period of 13 months. Rotavirus infection occurred throughout the year and peaked in March. The lowest rotavirus infection occurred in December.

Table 2. Genotype distribution of rotavirus in Accra, Ghana (Jan. 2004–Jan. 2005)

G type	P type						
	P[4]	P[6]	P[8]	P[9]	P mix	P nt	
G1	0	1	6	1	2	2	12 (17.1)
G2	1	3	4	0	4	4	16 (22.9)
G3	0	4	0	0	2	3	9 (12.9)
G9	0	7	4	0	0	1	12 (17.1)
G mix	2	2	0	0	0	1	5 (7.1)
G nt	0	10	1	0	2	3	16 (22.9)
TOTAL (%)	3 (4.3)	27 (38.6)	15 (21.4)	1 (1.4)	10 (14.3)	14 (20.0)	70 (100.0)

nt, not typable.

countries (14–18 months) and is probably due to the fact that rotavirus infection displays a distinct seasonal pattern in temperate climates, with epidemic peaks occurring in the cooler months of the year and being absent or uncommon during the warmer months of the year (19,22). In tropical areas, rotavirus disease has been reported to occur throughout the year with seasonal peaks occurring during the cool dry months of the year (8,20). Additionally, our data (Table 2) suggest that Ghanaian children are exposed to a wide range of different genotypes, probably more than what children in developed countries are exposed to. This may partly clarify the higher incidence of rotavirus disease among children in the developing world compared with those in the developed world.

The monthly distribution of rotavirus observed in this study is in accordance with findings reported previously (8,9). In this study, peak rotavirus infection (53.6%) was observed in March, however, the monthly infection peak is known to vary from year to year, and from country to country (23). No significant difference in the prevalence of rotavirus infection between male and female children was observed, which is also consistent with a previous report (24). On the other hand, several studies have reported significantly higher prevalence rates in men compared with women (25,26). It is difficult to explain why no significant gender differences in rotavirus infection were observed; we believe that this finding may be coincidental.

VP7 and VP4 characterizations were determined by molecular methods using genotype-specific primers. The major neutralization antigen VP7 is the primary target for neutralizing antibodies (27). VP4 determines virulence in humans; in addition, this structural protein elicits neutralizing antibodies (28). Sixty-eight percent of G types detected (G1, G2, and G3) were globally common, suggesting that the efficacy of the current rotavirus vaccine might be satisfactory in Accra, Ghana. G4, one of the most common worldwide G types (28), which also formed the basis for the development of the rotavirus vaccine, was detected only in one of the samples with dual infection. The most common rotavirus G type detected in this study was G2 (16/70), constituting 22.9% of all G types detected, followed by G9 and G1. The genotype distribution in the current study is slightly different from that reported by Enweronu-Laryea et al. in southern Ghana (10). Their study was conducted 3 years after the present study, and the main genotypes identified included G1 (50.9%), G2 (18.8%), G3 (12.8%), P[8] (36.1%), and P[6] (30.7%) (10). Evidence from both studies highlights the great diversity of rotavirus strains circulating in Ghana. Recent studies in other regions of the world have also documented other circulating serotypes. Studies in Brazil showed that a significant number of infections (60%) were caused by strains other than those contained in the vaccine (29,30). In Malawi, rotavirus strains with novel G/P combinations, such as G8P[6] and G8P[4] were identified in more than 42% of specimens examined (31).

Unusual genotypes and mixed infections such as G2P[8], G(2/3)P[6], G(3/4)P[4], and G(8/9)P[6] were observed in our dataset. The rotavirus genome consists of 11 segments of dsRNA. Two structural proteins, VP7 (encoded by the 7th, 8th, and 9th genes) depending on the strain and VP4 (encoded by the 4th gene) can segregate independently by the mechanism of reassortment to produce new rotavirus strains. Reassortment occurs because of the segmented nature of the rotavirus genome and can lead to unusual G and P types (32).

This is the first study to report on the burden of rotavirus disease and its genotypes in southern Ghana prior to the introduction of the rotavirus vaccine in the country. The findings of this study show a high burden of rotavirus disease among children in southern Ghana. The rotavirus strains causing gastroenteritis in children in southern Ghana are highly diverse and include unusual genotypes. Reinforcement of continuous surveillance of rotavirus types in Ghana is warranted.

This study has some limitations. First, only hospitalized patients were investigated and second, the stool samples were collected and investigated 10 years ago.

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