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Rotavirus as a cause of diarrhea among children under 5 years of age in urban Ghana: Prevalence and serotypes/genotypes

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Abstract

We collected clinical and morphological data from children with diarrhea attending 3 diverse hospital/clinics in Accra. Stool samples were tested for rotavirus and *Cryptosporidium* spp. 58% of the children with diarrhea had rotavirus infections and 25% of which were of the G3 sero/genotype. The most common strains were G3P[6] (18.8%) and G2P[6] (12.5%). *Cryptosporidium spp*. infections were uncommon (3/143, 2.0%).

Keywords

Rotavirus; child; diarrhea; Ghana; cryptosporidiosis; prevalence

Introduction

Diarrhea accounts for 25% of deaths in Ghanaian children <5 years of age, with >9 million episodes of diarrhea occurring annually (1). The financial loss to Ghana resulting from diarrheal diseases may be US$33 million a year of direct costs; indirect costs are considerably higher (1). Rotavirus (RV), enterotoxigenic *Escherichia coli* (ETEC), *Shigella*, *Salmonella*, *Campylobacter jejuni* and *Cryptosporidium spp*. are the most common known causes of diarrhea in developing nations such as Ghana.

There are currently no RV vaccines in common use in the extended program of immunization (EPI) in countries in sub-Saharan Africa except South Africa, though local RV epidemiology suggests a need (2). The complexity of the antigenic diversity of circulating rotavirus strains in West Africa will have implications for this vaccine. We studied RV and *Cryptosporidia spp*. prevalence in Ghanaian children brought to three outpatient clinics in Accra with a major complaint of diarrhea.

Materials and Methods

Parents and guardians of children <5 years of age at the out-patient-departments (OPDs, emergency rooms, and children's wards) of Maamobi Polyclinic, La General Hospital and the Princess Marie Louise Children's Hospital were recruited after obtaining written

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informed consent. The consent form was read to them in English or the language of choice (typically Twi, Ga and Ewe) in the presence of a witness. Only children <5 years of age with diarrhea defined as passage of ≥3 loose watery stools within a 24-hour period were eligible.

The study was approved by the ethics committees of Vanderbilt University and the Ministry of Health in Ghana. Consenting parents/guardians answered a 42-question study questionnaire. The stool samples were stored at the participating clinics at 4°C and transported weekly on ice to Noguchi Memorial Institute for Medical Research. The 143 samples were stored at −20°C until tested.

Laboratory Procedures

We tested stool samples for the presence of RV using an enzyme-linked immunosorbent assay (ELISA). Group A antigen detection was performed on 10% stool suspensions made in dilution buffer (RV IDEIA™ Dako diagnostic Ltd, Cambridgeshire, UK) as per manufacturer's instructions. The test results were read spectroscopically at a wavelength of 450nm. Each test plate had a negative and positive control.

ELISA-positive samples were characterized by RT-PCR. RNA was extracted from 200μL of 10% stool suspensions in 200μL of Bender buffer [0.1 M NaCl, 2M sucrose, 0.1M Tris-HCl (pH 8.0), 0.05M ethylenediaminetetracetic acid (EDTA) and 0.5% SDS] as described by Boom et. al (4). The samples were layered on a 12% resolving polyacrylamide gel and electrophoresed at 100V for 18 hours. The gels were visualized by the silver nitrate ($AgNO_3$) staining method (5). Samples that showed the expected electrophoretic patterns and thus had RNA of good integrity for further analysis were subjected to VP7 and VP4 typing by reverse transcriptase electrophoresis (RT-PCR).

For the RT-PCR, RNA was extracted from 500μl of 10% stool sample suspensions in phosphate buffered saline (PBS) by the phenol/chloroform method (6) and purified as earlier described (4). The purified RNA was reverse transcribed and genotyped using a semi-nested PCR (8). For VP4 typing, first round amplification of the VP4 gene was performed using the gene specific primer pair, Con2/Con3 (each 10pmol). The PCR master mix consisted of 10mM of each dATP, dCTP, dGTP and dTTP, 0.25 μL of *Taq* polymerase, 5× Green GoTaq® Reaction Buffer. This was followed by a second round genotyping polymerase chain reaction using VP4 specific primers (1T-1D, 2T-2, 3T-1, 4T-1 and 5T-1). Amplified products were analyzed on 2% agarose gel and genotyped.

For VP7 typing, the first round reaction was performed using gene specific primer pairs, Beg9 and End9 (each 2.5pmol) (9). This was followed by a second round reaction with VP7 genotype-specific primers (aBT-1, aCT-2, aET-3, aDT-4, aAT-8, aFT-9, G10, G12) and 10pmol of RVG9 in a reaction similar to the one described above. For both VP4 and VP7, 30 PCR cycles were performed with 1 min of denaturing at 94°C, 2 minutes of annealing at 42°C, 5 minutes of extension at 72°C and a final extension cycle at 72°C for 7 minutes.

Cryptosporidium spp. oocytes were detected using the Ziehl-Neelsen stained fecal smear (3). Specimens were stained with carbol fuschin, destained with 10% solution of sulfuric acid, washed and counterstained with methylene blue. Oocytes were then visualized under a light microscope (3).

Statistical Analysis

We entered data into a Microsoft® Access™ 2007 database and the data were analyzed using Intercooled Stata 8.0™ (Stata Corporation, College Station, TX, USA). The Z scores for weight for age were calculated using WHO Anthro™ (version 3, April 2009). Logistic

regression was used to evaluate risk factors that are associated with infection by RV in other published studies.

Results

Results were based on 143 of the 147 stool samples (97%) collected during the months of June through September 2009 (see Figure, SDC 1, which demonstrates the flow diagram of the Rotavirus study). The remaining 4 samples were not genotyped due to inadequate stool volume for ELISA testing and were thus excluded.

ELISA testing for RV identified 83/143 (58.0%) to be positive for RV group A. The highest prevalence (77.4%) was found in the 0–5 month age group (Table). The mean age of our 143 participants was 11.3 months (95% CI 9.9 – 12.6), with the median weight of 7.2 kg, interquartile range (IQR) of 2.7 kg and a range of 19.0 kg. Of the children with diarrhea who were positive for RV, 90.4% had vomiting and 45.8% had fever, compared with 60.0% with vomiting and 53.3% with fever in children who were negative for RV (p=0.001 for vomiting, p=0.09 for fever). Of RV-positive children with diarrhea, 39.8% had both vomiting and fever, compared with the 30.0% of those who were RV-negative (p=0.15).

Vomiting was more persistent and longer in duration in RV-positive children compared with RV-negative children (p=0.02). There was no significant difference between the baselines for mean diarrheal episodes per day, weight for age, mean diarrheal durations, and the mean fever durations between the RV-positive and -negative groups. In our logistic regression model, the greatest risk factor for presenting with RV was being within the 0–5 month age group (Table).

Rotavirus G3 genotype was the most common genotype determined (25.0%). Among the detected P types, P[6] was the most common (75.0%). The common strains identified strain were G3 P[6] (18.8%) and G2 P[6] (12.5%). Cryptosporidiosis was uncommon (3/143, 2%) and in one participant, a dual infection of RV and *Cryptosporidium spp.* was detected (see Figure, SDC 1, which demonstrates the flow diagram of the Rotavirus study). No children died.

Discussion

We found RV to be a common cause of diarrhea among children <5 years of age in 3 out-patient health facilities serving socioeconomically diverse groups of children in Accra, Ghana. Most diarrhea cases (58%) were caused by RV group A infections. As might be expected, the highest proportion of RV infections was found in the 0–5 month age group (77.4%) compared with 40.0% for older (18–48 months of age) children. RV-positive children had a longer duration of vomiting than RV-negative children, suggesting more severe gastrointestinal perturbations with RV than with other causes of diarrhea. A child between 0–5 months old was approximately 5 times more likely to be infected with RV, controlling for other factors, than an older child with diarrhea. Sex and nutritional status of a child did not predict RV infection.

The sero/genotype distribution of the RV group A strains in this study showed that G3 was the most common sero/genotype detected, followed by G2 and G1. This frequency follows the pattern reported from Ghana previously (7). Among the P genotypes, P[6] was predominant, followed by P[8] and the mixed genotypes P[6+4] and P[8+6]. In a previous report, P[4], which was not detected in this study, was shown to be the most common P genotype among children with gastroenteritis (7). The absence of P[4] genotypes in this study may be due to the fact that infecting RV genotypes vary yearly and by location (10).

RV infection is usually high during the relatively cool dry months with peak infections in February and low during the wet season in Ghana (10).

The presence of untypable G and P types could either be due to specific antigenic shifts in the primer binding sites, making present primers not suitable for typing, or they may represent new emerging strains. The common RV strains of G2P[6] (12.5%) and G3P[6] (18.8%) detected in our samples are unusual strains compared with previous work (7). This phenomenon could be explained by reassortments of the genomes of human RVs, potentiated by the fact that RVs are capable of co-infecting a single cell.

It is imperative that more studies determine the prevalent serotypes and genotypes in circulation to guide RV vaccine development in Africa and other developing countries. RV is a major cause of childhood diarrhea severe enough to require a clinic or hospital visit in urban Ghana and we believe an efficacious RV vaccine has the potential to make a major impact of mobility and mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table

Risk Factors Associated with Rotavirus Positivity in 143 Ghanaian Children

		N	RV Positive (%)	Odds Ratio (95% CI)	P-Value
Age	0–5	31	24 (77.4)	4.97 (1.3 – 19.4)	0.02
	6–11	58	31 (53.4)	1.45 (0.5 – 4.4)	0.51
	12–17	29	18 (62.1)	1.91 (0.5 – 6.8)	0.32
	18–48	25	10 (40.0)	1.0	
Sex	Female	52	34 (65.4)	1.0	
	Male	91	49 (53.9)	0.61 (0.3 – 1.4)	0.26
Weight for Age	Not Wasted ($Z \geq -2$)	80	50 (62.5)	1.0	
	Wasted ($Z < -2$)	51	28 (54.9)	0.68 (0.3 – 1.4)	0.31