Hospital-based Surveillance for Rotavirus Gastroenteritis in Children Younger Than 5 Years of Age in Ethiopia: 2007–2012

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Background: Rotavirus surveillance was initiated in Ethiopia to estimate the burden of rotavirus gastroenteritis in children <5 years of age, to generate data to assist the policy-making process for new vaccine introduction and to monitor impact of vaccination on disease burden after introduction.

Methods: Sentinel surveillance was conducted at 3 hospitals in Addis Ababa, Ethiopia using a standardized WHO surveillance protocol from August 2007 to March 2012. Children <5 years of age, hospitalized for the primary reason of treatment for acute gastroenteritis, were enrolled, stool samples were collected and tested for group A rotavirus using an enzyme immunoassay. Confirmed positive specimens were further characterized by rotavirus genotyping.

Results: A total of 1841 children were enrolled and 21% were rotavirus positive. Children 6–12 months of age had the highest proportion of rotavirus (36%) followed by children <6 months of age (23%). There was no significant difference between sexes. Significant differences in clinical characteristics, such as vomiting, vomiting episodes, cases with vomiting and diarrhea among rotavirus positive cases, were observed. Rotavirus circulated year round with peak prevalence from October through January. The most prevalent detected genotypes were G1P[8] (20%), G12P[8] (17%) and G3P[6] (15%), respectively.

Conclusions: Rotavirus infection is common in Ethiopian children. A safe and effective intervention against the infection is needed to prevent severity of the disease. Rotavirus vaccine introduction is planned before the end of 2013. The established surveillance system and the data generated can be used to monitor the impact of rotavirus vaccination program on severe disease.

Key Words: rotavirus, gastroenteritis, Ethiopean children

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Rotavirus infection is the leading cause of severe acute gastroenteritis in young children worldwide. Rotavirus disease is more severe than diarrhea caused by other enteric pathogens with symptoms including an average of 6 stools per day, severe dehydration, which is 14 times more frequent than in children without rotavirus diarrhea, vomiting and fever.[1–3] The administration of oral rehydration can be hampered by the accompanying vomiting.[4] Each year rotavirus infection leads to 453,000 rotavirus-related deaths globally with >85% of these deaths occurring in Africa and Asia.[4–8] In 2008, the World Health Organization (WHO) estimated that 28,218 deaths among children <5 years of age in Ethiopia were due to rotavirus diarrhea and rotavirus vaccines are not currently available.[8] Rotavirus vaccine introduction is planned for 2013.

Alhough bacterial and parasitic causes of gastroenteritis are relatively well studied, adequate information about viral etiologies of diarrhea is lacking in Ethiopia. However, some hospital-based studies in Addis Ababa and Jimma towns showed rotavirus as a major cause of nonbacterial acute gastroenteritis in infants and young children that accounted for 18–28% of acute gastroenteritis cases.[9–12]

Group A rotaviruses, which account for the vast majority of human disease, are classified into different P and G-types based on the 2 outer capsid proteins, VP4 and VP7, respectively. Studies have shown wide geographical variation in G- and P-type prevalence across continents, global temporal changes in the frequency of dominant strains and emergence of unusual P and G types and combinations.[13–18] On the basis of antigenic and genetic diversities, 27G types and 35P types have been identified to date among rotavirus strains of both human and animal origins.[19]

In sub-Saharan Africa, although the common human rotavirus strains (G1–G4 and G9) were observed, only G1, G2 and G9 were detected routinely, and the more unusual strains (G8 and P6) were detected more frequently than in other areas of the world.[20,21]

Ethiopia joined the African Rotavirus Surveillance Network in 2007 to conduct hospital-based surveillance and to estimate the burden of rotavirus gastroenteritis in children <5 years of age in 3 hospitals in Addis Ababa, Ethiopia. The evidence generated from the surveillance will assist policy makers to evaluate the need for rotavirus vaccination program for the country and to monitor the impact of vaccination following introduction. The objective of this paper is to describe the epidemiology and strain diversity of rotavirus in Ethiopia before vaccine introduction.

METHODS

Recruitment and Case Definitions

Rotavirus surveillance was started in August 2007 in Black Lion hospital and expanded to 2 more hospitals, Yekatit 12 in January 2008 and BeteZata in October 2011 in Addis Ababa, Ethiopia. Inclusion and exclusion criteria for diarrhea cases were applied as specified in the Regional Office for Africa standard operating procedures and WHO Generic Protocol.[22] Children <5 years of age who were hospitalized for the primary reason of treatment for acute gastroenteritis were eligible for inclusion. Hospitalization was generally defined as admission to a hospital ward; however, when a hospital bed was not available, children rehydrated in the emergency department (for at least 6 hours under observation) were also eligible. Exclusion criteria included bloody diarrhea, symptoms lasting 7 days before presentation to

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the hospital or acute gastroenteritis acquired during a hospitalization for another disease.

Specimen Collection

From each enrolled child, 2 g of diarrheal stool sample was collected in a labeled screw-top container within 48 hours after admission to a hospital ward for acute diarrhea and the samples were transported and stored at 4°C until testing at the National Reference Laboratory at the Ethiopian Health and Nutrition Research Institute. Additional demographic data (eg, age and sex) and information on clinical characteristics (eg, vomiting, diarrhea, vomiting episode within 24-hour cases with diarrhea and vomiting, temperature status, sunken eyes and lethargy) were also gathered using the standardized case report forms.

Laboratory Testing for Rotavirus

Diagnosis of rotavirus infection was determined by using an antigen capture enzyme immunoassay (EIA; IDEIA kit; DAKO Diagnostics, Denmark). All positives and 10% of specimens negative for rotavirus by EIA were retested at the regional reference laboratory by EIA as part of quality control. In addition, a total of 215 randomly selected rotavirus positive samples by EIA, which cover the entire period at each hospital, were further characterized at the rotavirus regional reference laboratory: MRC Diarrheal Pathogens Research Unit, MEDUNSA, South Africa. The samples were analyzed by polyacrylamide gel electrophoresis to determine the RNA migration patterns (long or short RNA patterns). To determine the rotavirus circulating strains in Addis Ababa, Ethiopia, the samples were subjected to reverse-transcriptase polymerase chain reaction and semi-nested multiplex polymerase chain reaction assays to identify G and P types using previously published primers.[23–25] Sixteen randomly selected VP4 and VP7 amplicons were sent to Inqaba Biotechnology Industry (Pty) Ltd, Pretoria, South Africa for sequencing to confirm the genotyping results. The amplicons were sequenced using the dideoxynucleotide chain termination method with an ABI 3130XL sequencer. The sequences were verified by BLASTN analysis on the National Centre for Biotechnology Information website. Multiple alignments were performed by BioEditand Mafft software packages. The dendrograms were constructed using neighbour-joining method with MEGA version 5.1 software.[26]

Data Capture and Analysis

Data generated from August 2007 to March 2012 were regularly submitted to the Federal Ministries of Health of Ethiopia and to the WHO country office, which later sent data to the WHO Regional Office for Africa. Data analysis was performed using Epi-Info 2002 software (Centers for Disease Control and Prevention) to determine the prevalence of rotavirus infection among recruited children of <5 years of age who were hospitalized with acute gastroenteritis, age distribution, seasonal patterns of rotavirus disease among recruited children and characterization of rotavirus strains circulating in Addis Ababa, Ethiopia.

RESULTS

In the 3 sentinel sites, Black Lion, Yekatit 12 and BeteZata hospitals, a total of 1841 fecal specimens were collected from children of <5 years of age who were hospitalized with acute gastroenteritis from August 2007 to March 2012. Rotavirus positive cases distribution by year of hospital admission and the 3 surveillance sites are shown in Table 1. Based on the surveillance period of each hospital, a total of 233 (20%), 120 (19%) and 35 (81%) positive cases were observed, respectively. Unlike the other hospitals, the observed high rate of rotavirus positive cases at BeteZata hospital is because surveillance was only conducted for 6 months (October 2011 through March 2012) during the peak of rotavirus activity.

Rotavirus infection was detected in 21% (388/1841) of children that presented with acute diarrhea. The age group analysis of rotavirus

TABLE 1. Acute Diarrhea Cases by Year of Hospitalization and Rotavirus Positive Cases Distribution During the Surveillance Periods of the 3 Surveillance Sites, August 2007–March, 2012

Year of Surveillance	Black Lion Hospital		Yekatit 12 Hospital		BeteZata Hospital	
	No of Specimen Collected	No of Rotavirus-positive Cases n (%)	No of Specimen Collected	No of Rotavirus-positive Cases n (%)	No of Specimen Collected	No of Rotavirus-positive Cases n (%)
2007*	60	36 (60)	—	—	—	—
2008†	181	35 (19)	28	12 (43)	—	—
2009	231	40 (17)	223	29 (13)	—	—
2010	324	64 (20)	208	36 (17)	—	—
2011‡	318	48 (15)	128	31 (24)	16	14 (88)
2012§	45	10 (21)	52	12 (23)	27	21 (78)
Total	1159	233 (20)	639	120 (19)	43	35 (81)

*Surveillance started at Black Lion hospital in August 2007.
†Surveillance started at Yekatit 12 hospital in January 2008.
‡Surveillance started at BeteZata hospital in October 2011.
§Surveillance conducted from January to March of 2012 at all sites.

TABLE 2. Prevalence of Rotavirus Infection by Age Group and Sex of Children With Acute Diarrhea Cases at the Three Surveillance Sites, August 2007 to March 2012

Age in Months*	Total Number of Specimen Tested	Rotavirus-positive Cases n (%)	Rotavirus-negative Cases n (%)	*P* Value
<6 Months	244	57 (23)	187 (77)	
6–12 months	448	161 (36)	287 (64)	
>12 to < 24 months	500	111 (22)	389 (78)	
24 to < 60 months	649	59 (9)	590 (91)	
Total	1841	388 (21)*	1453 (79)	<0.001

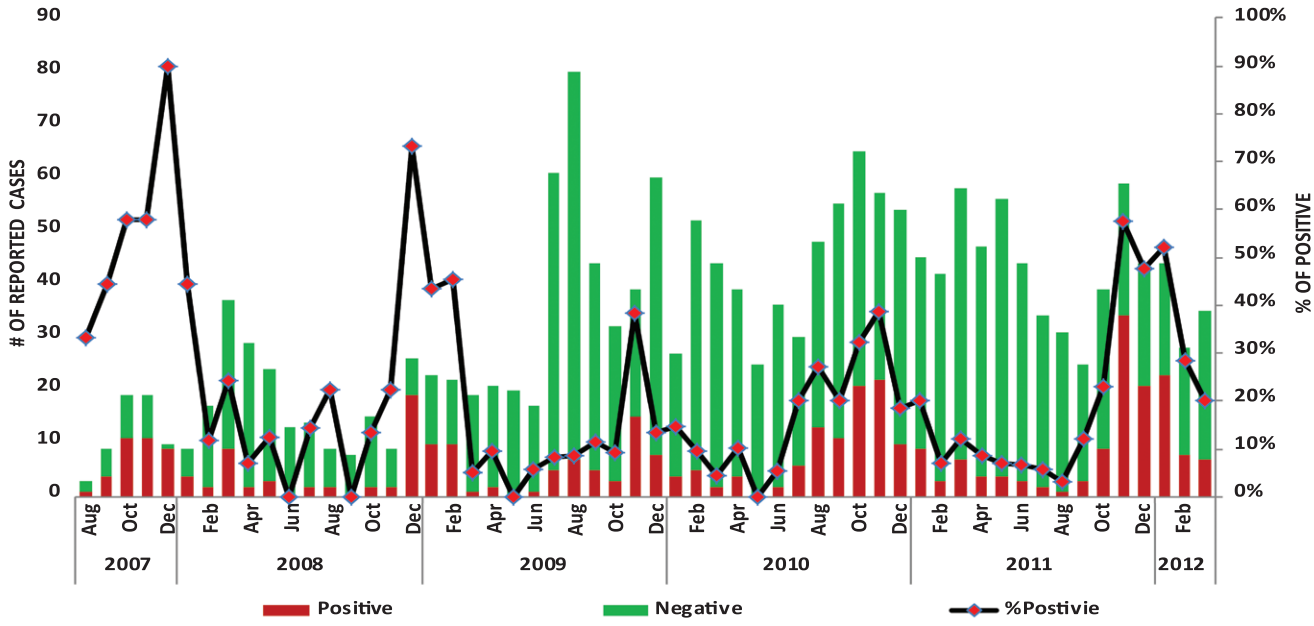

FIGURE 1. Seasonal distribution of rotavirus during the surveillance periods, August 2007 to March 2012.

positive children with diarrhea manifested the highest infection rate between 6 and 12 months of age with highest proportion of rotavirus positive specimens (36%) followed by children <6 months of age (23%) and children between the age of >12 months and <24 months of age (22%).Children <24 months of age accounted 85% of the total rotavirus positive specimens detected. Overall, significant differences in proportion of rotavirus positive cases were found among the 4 age groups, ($P < 0.001$; Table 2). There was no significant difference in rotavirus infection between male and female children ($P = 0.55$; Table 2).

Significant differences of clinical characteristics in the proportion with vomiting ($P = 0.0002$), vomiting episodes within 24 hours ($P = 0.0008$) and the proportion of cases with vomiting and diarrhea among age groups of rotavirus positive cases ($P = 0.0003$) were observed. The features of these clinical characteristics are observed to be higher in the age groups of children <2 years than with the age group of > 2 years. There was no significant differences in positive cases of all age groups with the proportion of an elevated temperature ($P = 0.13$), sunken eyes ($P = 0.126$), lethargy ($P = 0.20$) and sex ($P = 0.55$).

Seasonality of rotavirus infections varied across the surveillance period reflecting the difference in the climatic conditions. Even though rotavirus was circulating year round, the prevalence of rotavirus infection peaked from October through January (Fig. 1).

Between 2008 and 2011, 215 rotavirus positive samples were characterized for both G and P types. The most prevalent genotypes detected during this period were G1P[8] (20%), G12P[8] (17%), G3P[6] (15%) and G2P[4] (11%), respectively. Rotavirus genotypes G1P[6], G9P[8], G9P[6], G2P[6] and G12P[6] were detected with lower prevalence. Mixed infections occurred in 11% of cases (8 mixed G-types and 16 mixed P-types) and 5% of specimens had either partially or untypeable G or P strains. The distribution of G and P types circulating in Addis Ababa, Ethiopia found over 4 years period (2008–2011) is described in Table 3. Majority of the strains circulated in 2008 were G2P[4] (31%) and G1P[8] (15%), while in 2009, there was an increase in number of the G1P[8] and G3P[6] rotavirus strains accounting for 32% and 22%, respectively. In 2010, the G12P[8] (21%) and G1P[8] (16%) were predominant strains detected and the level of the G12P[8] (36%) and G1P[8] (20%) continue to increase in 2011.

Predominantly long RNA migration profiles were observed in most of the characterized samples (53.9%), while short RNA pattern was detected at 36.2%. Among the predominant strains that were detected, G1P[8] and G12P[8] strains were strongly associated with the long electrophoretic pattern while the G3P[6], G2P[4] and G2P[6] strains were associated with the short electrophoretic pattern.

Phylogenetic and sequence analyses of the Ethiopian G1 rotavirus strains showed genetic variability among the strains and the strains clustered into 2 separate genetic lineages (lineages I and II) and the strains were more closely related to the strains from Germany (Ger109-08, GU392988), Canada (THY126-05, JX470511), Belgium (Be1175-09, JN849154) and Russia (H308-04,GU377135), respectively. The highest nucleotide identity level among the Ethiopian strains and the reference strains ranged from 93% to 99%.The G3P[6] showed a steady increase during the study period from 12% to 22%, and the sequenced strains displayed 99% nucleotide and amino acid identity level with other Ethiopian reference strain (ETH44, JF909038). The Ethiopian strains formed independent cluster in lineage III when compared with the G3 strains from other countries. The G12 rotavirus strains exhibited

TABLE 3. Distribution of the G and P Genotypes Circulating in Addis Ababa, Ethiopia, 2008–2011

	2008	2009	2010	2011	Total
G1P[8]	8 (15%)	16 (32%)	9 (16%)	11 (20%)	44 (20%)
G12P[8]	4 (8%)	1 (2%)	12 (21%)	20 (36%)	37 (17%)
G3P[6]	6 (12%)	11 (22%)	8 (14%)	8 (15%)	33 (15%)
G2P[4]	16 (31%)	3 (6%)	4 (7%)	0	23 (11%)
G1P[6]	5 (10%)	4 (8%)	5 (9%)	1 (2%)	15 (7%)
G9P[8]	4 (8%)	2 (4%)	3 (5%0	0	9 (4%)
G9P[6]	0	4 (8%)	3 (5%)	1 (2%)	8 (4%)
G2P[6]	7 (13%)	0	0	0	7 (3%)
G12P[6]	0	0	2 (3%)	2 (4%)	4 (2%)
Mixed G	0	1 (2%)	1 (2%)	6 (11%)	8 (4%)
Mixed P	2 (4%)	5 (10%)	4 (7%)	5 (9%)	16 (7%)
Partially G/P	0	3 (6%)	7 (12%)	1 (2%)	11 (5%)
No of strains	52 (24)	50 (23)	58 (27)	55 (26)	215 (100)

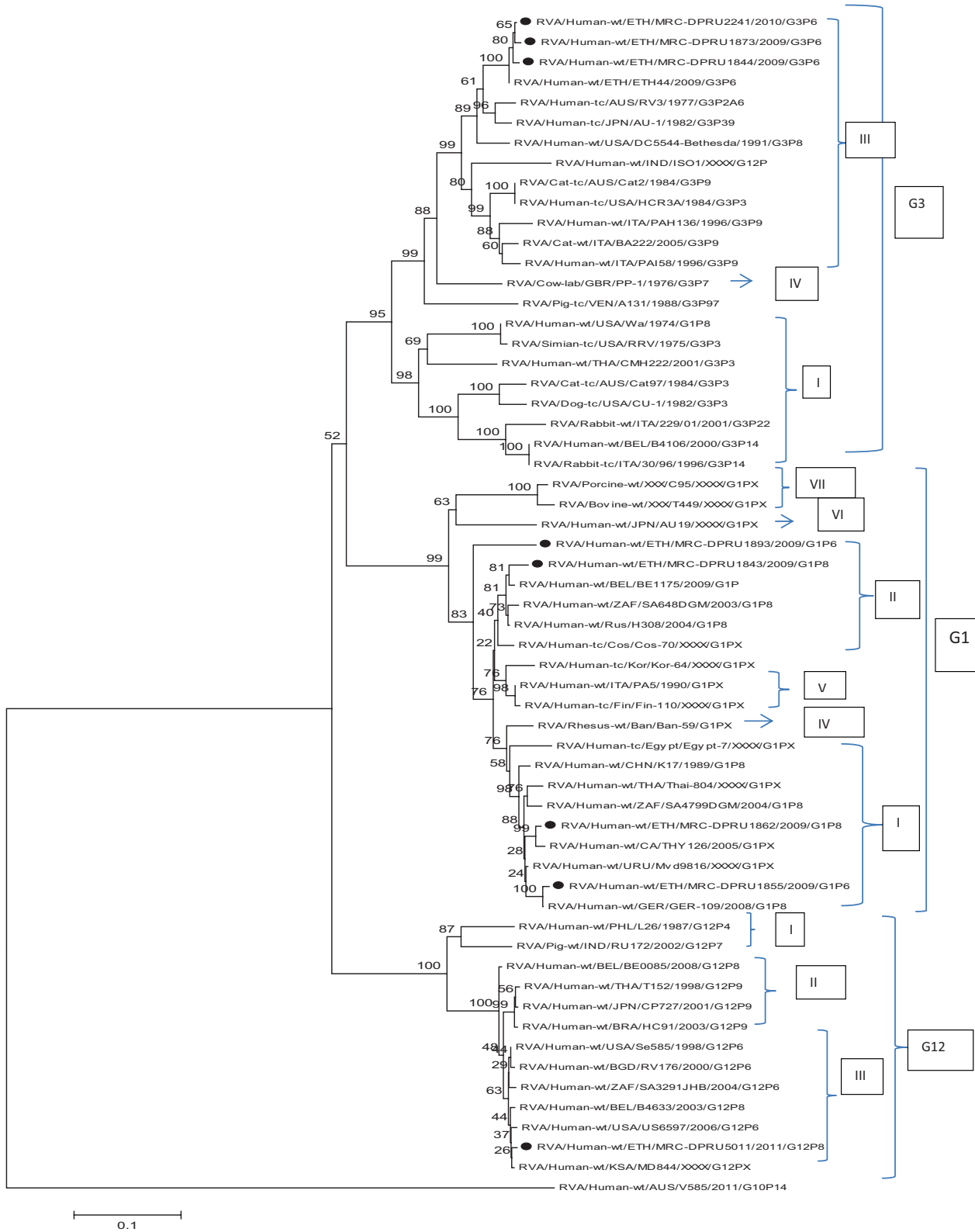

FIGURE 2. Phylogenetic tree representing 59 rotavirus strains G1, G3 and G12 (Ethiopian strains indicated in black solid dots).The evolutionary history was inferred using the MEGA version 5.1, neighbour-joining method. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The percentage of replicate trees in which the strains clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values <50% are not shown on tree.

98% nucleotide homology with the strains from Belgium (B463-03, JN849116) and clustered in lineage III branch (Fig. 2).

DISCUSSION

Since Ethiopia joined the African Rotavirus Surveillance Network in 2007, 1841 hospitalized children<5 years of age presenting with acute diarrheal illness were enrolled. Similar to previous reports of rotavirus diarrhea in Ethiopia which were found with a prevalence of rotavirus from 18% to 28%,[9–12] 21% of children with acute gastroenteritis in this study were infected with rotavirus in the 3 hospitals during the surveillance period. Overall, the highest prevalence (36%) of rotavirus infections occurred in children 6–12 months of age with the majority of all rotavirus infections occurring in this age group. Children <2 years of age accounted 85% of the total rotavirus positive specimens detected. Similar to other studies,[12,27] our findings confirm high incidence of rotavirus gastroenteritis during the first 2 years of life and, hence, a need for long-term protection induced by rotavirus vaccination.

No significant difference in rotavirus infection was observed between the 2 sexes although age was found to be significantly associated with rotavirus infection as described before.[10,12] Similar with other studies, significant differences of clinical characteristics such as vomiting, >5 times of vomiting episodes within 24 hours and both diarrhea and vomiting were observed among the different age groups of rotavirus positive cases, respectively.[3,5,12]

As reported before, rotavirus infections occurred throughout the surveillance period and peak prevalence was observed in autumn, from October through January, following the heavy rainy months.[10,11] This seasonality of rotavirus disease is unlike that was observed elsewhere in Africa. In Kenya, seasonal peaks are observed during the dry seasons.[28–30] In Southern African countries, rotavirus occurs in autumn and winter seasons but not in dry seasons. In Western African countries, rotavirus occurs during the dry cool months.[31–35]

The most prevalent genotypes observed during the surveillance period was G1P[8] (20%) like most African countries,[18,34] followed by G12P[8] (17%) and G3P[6] (15%). According to rotavirus surveillance conducted in 8 African countries during 2006–2008, G1P[8] was the most frequently detected (22%) genotype except in Cameroon and Zimbabwe where G9P[8](19%) and G12P[6] (18%) were the most frequently detected genotypes.[18]The observed unusual types in this surveillance, G12P[8] and G3P[6], might be due to potential reassortants between human and animal rotavirus strains as a result of zoonotic infection. The emergence of these new strains was also observed in Kenya, Ethiopia, Cameroon and Togo and may represent dynamic molecular events occurring in Africa, generating rotavirus reassortants.[18,35–37]

Mixed infections occurred in 11% of cases (8 mixed G-types and 16 mixed P-types) as have been observed elsewhere.[18,38] In countries with temperate climates, the most prevalent rotavirus G/P genotype combinations were G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8].[35,36] Low number of (5%) specimen with either partially or untypeable G or P strains were observed compare to previous study in Africa where an exceptionally high number of untypeable rotavirus strains (mean, 30%; range, 10%–43%) were recorded.[18] It is possible that these untypeable strains represent unusual rotavirus strains of animal origin.[35,36] Alternatively, they could be the result of the primers used for typing by reverse-transcriptase polymerase chain reaction that do not represent consensus sequences in these animal derived rotavirus strains.[18]

In addition to the predominant long electrophoretype RNA profiles, G3P[6] strains were found with short electrophoretic pattern that formed an independent cluster in lineage III similar with Togo isolates.

There are limitations in these surveillance activities; first, the current rotavirus surveillance study sites were located in urban Addis Ababa and the generated data may not be representatives of the prevalence of rotavirus in rural settings or other urban areas in Ethiopia. Expansion of surveillance sites to rural areas should be considered to understand the true burden of the disease nationally. Second, not all collected rotavirus-positive specimens were genotyped and may not be representatives of circulating strains during the surveillance period. However, it is believed that the predominant strains were captured and their diversity was demonstrated in Addis Ababa, Ethiopia.

In conclusion, these data show rotavirus infection is common in Ethiopian children and hence the need for safe and effective interventions against rotavirus infection to prevent severe disease. Introduction of rotavirus vaccine is planned to be implemented in 2013 in Ethiopia. It is important to monitor the diversity of rotavirus strains. The established surveillance system and the data generated can be used to monitor the impact of a rotavirus vaccination program on severe rotavirus disease in Ethiopia.

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