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Research paper

Molecular epidemiology of rotaviruses in Northwest Ethiopia after national vaccine introduction

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ABSTRACT

Background: Rotaviruses mortality among infants and young children is high in Sub-Saharan Africa. Recently, Ethiopia introduced the monovalent rotavirus vaccine in its national immunization program to decrease the burden of rotavirus disease and mortality. Rotavirus surveillance in Ethiopia is based largely on data provided by sentinel hospitals in its capital Addis Ababa.

Objective: To assess rotavirus abundancy and diversity in outpatient infants and children outside of Addis Ababa in the early post-introduction period.

Method: Fecal samples were obtained from children aged less than five years presenting with diarrhea at outpatient health institutions in two cities in Northwest Ethiopia, Gondar and Bahir Dar, from November 2015 to April 2016. Basic demographic data were assessed. Real-time RT-PCR was used to detect rotavirus A RNA. Based on sequences of VP4 and VP7 gene segments phylogenetic analysis was performed.

Results: Rotavirus wildtype positivity was 25% (113/450). Rotavirus infection was less common in infants below 6 months than in children of all other age-groups. Rotavirus genotype distributions were distinct between Bahir Dar and Gondar. In total, wildtype G3P[8], G2P[4], G9P[8], G12P[8], and G3P[6] rotaviruses were detected in 68 (60.2%), 21 (18.6%), 13 (11.5%), 9 (8.0%), and 2 (1.8%) of the positive samples, respectively. Wildtype G1P[8] strains were absent. The phylogenetic analysis revealed close relatedness of current rotaviruses with Ethiopian strains of the pre-vaccination period.

Conclusion: In the early period after the introduction of vaccination, rotaviruses in Northwestern Ethiopia were frequent in children of 6–59 months and diverse. High phylogenetic relatedness with strains of the pre-vaccine era, indicate absence of early vaccine-induced strain replacement. Future surveillance studies should be carried out throughout the country to gain comprehensive data on rotavirus strain diversity and to monitor the effect of the ongoing vaccine program on the disease burden and eventual rotavirus strain replacement.

1. Introduction

Diarrheal diseases are the second leading cause of global morbidity and their mortality is highest in early childhood in resource-limited countries (G. B. D. Diarrhoeal Diseases Collaborators, 2017). Globally, about 453,000 annual child deaths occur due to rotavirus infections. Thereof, 56% involve Sub-Saharan Africa and 3% Ethiopia (Tate et al., 2016). Rotavirus infections display a distinct seasonal pattern in temperate climates, but in countries with tropical climates, rotavirus disease seasonality is less defined (Esona and Gautam, 2015). In Ethiopia, rotavirus circulates year round with peak prevalence during dry season from October through January (Abebe et al., 2014; Mwenda et al., 2010).

Rotavirus, a non-enveloped double-stranded RNA virus, belongs to the family of *Reoviridae*. The double-stranded RNA genome is organized in 11 segments. These RNA segments encode six structural proteins (VP1-VP4, VP6, and VP7) and five or six nonstructural proteins (NSP1-NSP6) (Esona and Gautam, 2015). On the basis of variation of the nucleotide sequences of VP6, nine rotavirus species are referred as A to I (Lefkowitz et al., 2018). More than 90% of human rotavirus infections are due to rotavirus A (Esona and Gautam, 2015). Genes encoding the outer capsid proteins, VP7 and VP4, are routinely used for basic genotyping of the virus into G-types and P-types, respectively (Esona and Gautam, 2015). To date, 36 G genotypes and 51 P genotypes of rotavirus A have been described in various hosts (Esona and Gautam, 2015). Among them, G genotypes G1, G2, G3, G4, G9 and G12 and P genotypes

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P[4], P[6] and P[8] are most frequently reported in humans (Doro et al., 2014).

In November 2013, Ethiopia included Rotarix in its national immunization program to decrease the burden of rotavirus morbidity and mortality (Mwenda et al., 2017). Rotarix is a monovalent attenuated G1P[8] rotavirus strain and two doses are orally administered (Vesikari et al., 2004). According to the Ethiopia Immunization Implementation Guideline, the first dose of the vaccine is given at the age of six weeks and the second dose at week ten (Federal Ministry of Health [Ethiopia], 2015). Rotavirus vaccination coverage in Ethiopia was estimated at 63% in 2014 and 83% in 2015 (Weldegebriel et al., 2017) but varies across the country (Central Statistical Agency [Ethiopia] and ICF, 2016). In Northwest Ethiopia, the proportion of rotavirus vaccinated children was estimated at 63% to 76% in 2015 and 64% in 2016 (Azage et al., 2016; Feleke et al., 2018). To evaluate the impact of rotavirus vaccination, Ethiopia joined the African Rotavirus Surveillance Network (ARSN). However, sentinel surveillance is restricted to hospitals in the capital, Addis Ababa (Mwenda et al., 2017). To evaluate the necessity of expanded post-introduction surveillance, the present study assessed rotavirus diversity in outpatient children outside the capital.

2. Materials and methods

2.1. Study area, design and period

The present cross-sectional study was conducted at outpatient health institutions in Gondar and Bahir Dar, Northwest Ethiopia from November 2015 to April 2016 (Fig. 1). Bahir Dar is the capital of Amhara Regional State situated at the southern shore of Lake Tana, in 320 km distance from Addis Ababa. Gondar is an ancient city and the current administrative center of the North Gondar Zone of the Amhara Regional State, 420 km apart from the capital. The study was approved by the Ethical Review Board of the University of Gondar (O/V/RC/05/1180/2016). Parents or guardians of children provided their signed informed consent to participate in the study.

2.2. Specimen collection, transportation and processing

Children under five years of age, who seek outpatient treatment because of acute diarrheal disease at selected health institutions during the study period, were included in the study. A case of diarrhea was defined as the passage of three or more loose or watery stools during the previous 24 h. Children's sex, age and area of residence were assessed. Data from certificates of vaccination were not available. Parents or guardians of children were properly instructed on how to collect the fecal sample of their child. In total, 450 children were included in the study and one fecal specimen per child was collected and stored at 4 °C at the collection sites until transported for long time storage at −20 °C.

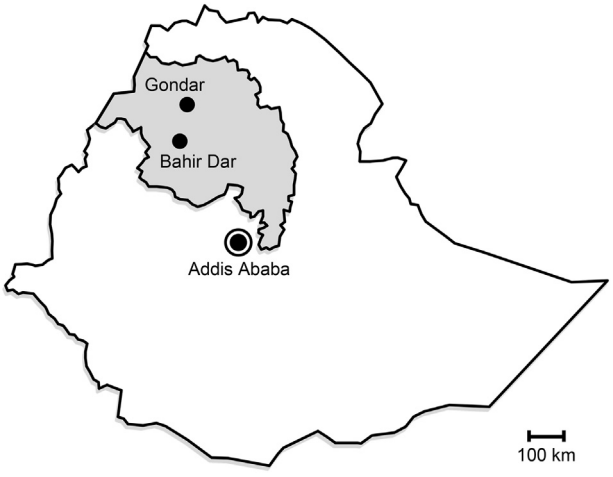

Fig. 1. Map of the study area and sampling sites. Stool samples were obtained from Gondar and Bahir Dar in the Amhara Regional State of Ethiopia.

2.3. Rotavirus detection and characterization

Species A rotaviruses were detected and molecularly characterized as described previously (Pietsch et al., 2011). Briefly, samples were screened for the presence of rotavirus A RNA by real-time RT-PCR. Detected rotavirus strains were G and P genotyped by phylogenetic analysis of obtained VP7 and VP4 nucleic acid sequences. Phylogenetic trees were constructed with MEGA software version 5 by the Maximum-Likelihood method. Bootstrap analysis was performed with 1000 replicates (Tamura et al., 2011). Representative VP7 and VP4 sequences from each sampling site were submitted to GenBank (accession numbers MH382838-MH382871).

2.4. Data analysis

Data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) software version 16. Categorical variables were compared using chi-square test. Significance of age distribution was assessed by Fisher's exact test. P values of < 0.05 were regarded as statistically significant.

3. Results

3.1. Characteristics of study participants

In total, 450 children were enrolled in the study. Of these, 200 (44.4%) were from Gondar and 250 (55.6%) from Bahir Dar. Thereof, 120 children lived in rural and 330 children in urban areas. The proportion of enrolled children from rural areas was 28.5% in Gondar and 25.2% in Bahir Dar. The majority of children were one or two years old (Table 1). The proportion of girls was 222 (49.3%).

3.2. Rotavirus detections and genotyping

Rotavirus wildtype RNA was detected in 113 stool samples (25.1%, 95% CI 21.3%–29.3%). Testing was more frequently positive in samples from Gondar ($n = 68$, 34%) than from Bahir Dar ($n = 45$, 18%) ($P = .0001$). Rotavirus positivity was equal among boys and girls and among samples from rural and urban children. Rotavirus peak activity was observed from January to March in Gondar and January to April in Bahir Dar (Fig. 2). The proportion of rotavirus wildtype positive samples was lower in children aged below 6 months but equally distributed in older children (Table 1).

For all rotavirus positive samples G and P genotyping were successful. In Gondar, G3P[8] rotaviruses prevailed sporadic G9P[8] and G12P[8] strains. In Bahir Dar, five different rotavirus genotypes, G2P[4], G9P[8], G3P[6], G3P[8] and G12P[8] were found co-circulating throughout the sampling period with the first two being prevalent

Table 1
Rotavirus wildtype infections by age group.

Age (months)	Rotavirus positive cases n (%)	Rotavirus negative cases n (%)	Total number of specimens tested n (%)	P value
< 6	4 (10.8)	33 (89.2)	37	0.046
6 to < 12	15 (25.9)	43 (74.1)	58	0.9
12 to < 24	36 (25.7)	104 (74.3)	140	0.9
24 to < 36	22 (21.6)	80 (78.4)	102	0.4
36 to < 48	19 (28.8)	47 (71.2)	66	0.4
48 to < 60	17 (36.2)	30 (63.8)	47	0.1
Total	113 (25.1)	337 (74.9)	450	