BRIEF COMMUNICATION

DETECTION OF ROTARIX VACCINE VIRUSES IN OLDER CHILDREN WITH ACUTE GASTROENTERITIS, NORTHWEST ETHIOPIA

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ABSTRACT

Background: *In 2013, Ethiopia introduced in its national routine immunization program vaccination against rotavirus infection to reduce morbidity and mortality. Excretion of rotavirus vaccine has been reported in fecal samples of vaccinated and unvaccinated children with asymptomatic and symptomatic infections. However, there is paucity of information regarding the circulation of rotavirus and its vaccine strains in Ethiopia.*

Objective: *This study was carried out to investigate the presence and circulation of rotavirus vaccine strains.*

Method: *From November 2015 to April 2016, a total of 450 fecal samples were collected from under-5 children with diarrhea in Gondar and Bahir Dar for detection and genotyping of enteric virus. Basic demographic data were assessed. All stool samples were screened for rotarix vaccine using real-time PCR and sequencing for molecular typing. Phylogenetic analysis and online genotyping tools were used to analyze the sequences.*

Result: *During rotavirus surveillance study at outpatient health institution in Northwest Ethiopia, live-attenuated Rotarix vaccine virus was detected in 12/450 (2.7%) of diarrheic children below five years of age. The vaccine virus copies detected in the patients reached up to $x10^9$ genome equivalents per gram of stool. Concurrent infections with norovirus, adenovirus, or human parechovirus were frequent.*

Conclusion: *This finding highlights the need of surveillance of vaccine derived rotavirus infection in children.*

Keywords: *rotavirus, gastroenteritis, genotype, diarrhea, vaccine strain, childhood*

INTRODUCTION

In resource-limited countries, rotaviruses rank highest among the viral causes of severe gastroenteritis and mortality associated with the disease (1). Rotaviruses are triple-layered and non-enveloped viruses with RNA genomes that belong to the *Reoviridae* family. Its genome is organized in 11 segments. The virus encodes six structural proteins (VP1-VP4, VP6, and VP7) and five or six nonstructural proteins (NSP1- NSP6) (2). On the basis of variation of the nucleotide sequences of VP6, nine rotavirus species are referred as A to I (3). More than 90% of human rotavirus infections are due to rotavirus A (2). The VP7 and VP4 genes are routinely used for basic genotyping of the virus into G-types and P-types, respectively (2).

In 2006, two rotavirus vaccines, Rotarix (RV1) and RotaTeq (RV5), were licensed. RotaTeq (Merck & Co. Inc) contains five human and bovine rotavirus reassortants(4). Rotarix (GlaxoSmithKline) is an oral monovalent attenuated G1P[8] strain administered orally (4). The World Health Organization has recommended vaccination against rotavirus since 2009.

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This led to its introduction in more than 100 countries (5).

Rotavirus vaccine was introduced in Ethiopia with the aim of decreasing the burden of rotavirus-associated morbidity and mortality in November, 2013(6). In routine immunization practice in Ethiopia, the first dose of the vaccine is given when the child is six weeks of age and the second dose at week ten (7). In 2015, the average vaccination coverage reached 83% in the country (8). In 2016, vaccination coverage in Northwest Ethiopia was 64%, whereas in 2019 it became 76.6% (9, 10, 11).The efficacy of the vaccine is significantly different in developed (85%) and developing (<50%) countries (12). However, rotavirus was still frequent in children of 6–59 months with diverse genotypes reported after the introduction of the vaccine in Northwest Ethiopia in 2018 (13). Nevertheless, the burden of viral diarrhea declined in Africa (6).

Despite the success of vaccination programs, concerns regarding the spread of vaccine viruses require further investigation. As does the wild-type rotaviruses, vaccine virus replicates in the intestine of vaccinated infants and is excreted in feces (14). Horizontal transmission of vaccine virus among close contacts has been reported (15–17).This study identified rotavirus vaccine strains in diarrheic children in Northwest Ethiopia.

METHOD

Study area and population: From infants and children below the age of 5 years with diarrhea, a total of 450 fecal samples were collected at outpatient health institutions in Northwest Ethiopia during November 2015 through April 2016 primarily for rotavirus surveillance(13). This study was part of the rotavirus surveillance program for which details on the study area, period, and design were previously described (13).

Nucleic acid extraction: For enteric virus genotyping, 10% suspensions of fecal samples were prepared in 1% phosphate buffered saline (PBS, pH 7.2). Adenovirus DNA was extracted with MagNA Pure 96 Instrument (Roche, Germany) as per the instruction of manufacturers.

Detection and genotyping of Adenovirus: All fecal samples were initially screened for the presence of rotavirus with RT-PCR as described previously (18). Those samples positive for rotavirus vaccine were further tested for the presence of norovirus, adenovirus, astrovirus, sapovirus, and parechovirus by real time (RT)-PCR.

Sequencing and Phylogenetic analysis: The PCR product was subjected to electrophoresis in a 2% agarose gel and purified with Wizard® SV Gel and PCR Clean-Up System (Promega, Mannheim, Germany). A sequencing reaction was performed by using BigDye™ Terminator v3.1 Cycle Sequencing (Applied Biosystems Corporation, Foster City, CA, USA). Sanger sequencing of the rotavirus VP4 and VP7 gene segments was carried out as previously described (19). Phylogenetic trees were constructed using Mega software (version 7). The Neighbor-Joining (NJ) method under Kimura's two-parameter correction was selected to construct trees. Bootstrap analysis was performed with 1,000 replicates. Representative sequences for both the VP4 and VP7 gene segments were deposited in the GenBank under the accession numbers MH884609-MH884610.

Statistical analysis: Statistical analysis was performed using SPSS version 16.0 software. Chi-square testing was used to determine the association between the dependent and the different demographic variables. A P-value of less than 0.05 was considered statistically significant.

RESULT

Rotarix vaccine virus was detected in 12 out of 450 diarrheic children (2.7%). The age of the children positive for vaccine virus ranged from 1 to 28 month. The majority of the children were boys (9/12) and residents in urban areas (8/12). The viral copy number per gram of stool and the threshold cycles (C_T) were determined with RT-qPCR. The C_T values ranged from 19 to 40 cycles. The lowest and the highest C_T corresponded with viral copies of 7.62 x 10^9 and 8.00 x10^3 genomic equivalents per gram of the stool, respectively. Seven of the children had concurrent infection with norovirus (2/12), adenovirus (5/129), or human parechovirus (2/12) (Table 1).

Sanger sequencing and phylogenetic analysis of the VP4 and VP7 gene segments confirmed the presence of Rotarix vaccine type G1P[8]virus. Analysis of the VP7 sequences of the vaccine strains revealed 100% nucleotide identity within one another and shared the highest phylogenetic relatedness with previously published vaccine sequences in the United States America from 1988 and 2009 and another vaccine sequence reported from Japan. The current vaccine virus sequences were distinct from previously reported wild type G1 sequences from Ethiopia and clustered in separate lineages (Fig.1).

The VP4 sequences also confirmed identical nucleotide relatedness within each other and clustered with previously published sequences of Rotarix vaccine strains from the United States of America. The VP4 vaccine sequences are also distinct from previously reported wild type VP4 gene sequences from Ethiopia (Fig. 2).

Table 1: Rotavirus vaccine strains isolated from diarrheic children, viral load, child information, and year of collection, Northwest Ethiopia

No	Rotavirus vaccine strain	C_T value	Month of collection	Resi-dence	Age in months	Sex	Concurrent viral infection	
							Viruses	C_T value
1	RVA/Vaccine/ETH/Rtarix-BD396/2015/G1P[8]	33.3	December	Urban	8	Male	None	-
2	RVA/Vaccine/ETH/Rtarix-BD435/2016/G1P[8]	22.5	January	Urban	24	Male	HAdV HPeV	35.2 31.1
3	RVA/Vaccine/ETH/Rtarix-BD436/2016/G1P[8]	36.0	January	Rural	17	Female	NoV	25.0
4	RVA/Vaccine/ETH/Rtarix-BD437/2016/G1P[8]	25.0	January	Rural	8	Male	HAdV	37.6
5	RVA/Vaccine/ETH/Rtarix-BD439/2016/G1P[8]	31.0	January	Urban	11	Male	HAdV	40.0
6	RVA/Vaccine/ETH/Rtarix-BD444/2016/G1P[8]	34.7	January	Rural	28	Male	HAdV NoV	39.6 24.9
7	RVA/Vaccine/ETH/Rtarix-BD445/2016/G1P[8]	19.0	January	Urban	1	Male	HAdV	34.6
8	RVA/Vaccine/ETH/Rtarix-BD476/2016/G1P[8]	40.0	February	Urban	16	Male	HPeV	30.8
9	RVA/Vaccine/ETH/Rtarix-BD498/2016/G1P[8]	30.7	February	Rural	25	Male	None	-
10	RVA/Vaccine/ETH/Rtarix-BD506/2016/G1P[8]	37.0	February	Urban	18	Male	None	-
11	RVA/Vaccine/ETH/Rtarix-BD509/2016/G1P[8]	32.0	February	Urban	9	Female	None	-
12	RVA/Vaccine/ETH/Rtarix-BD512/2016/G1P[8]	32.1	February	Urban	13	Female	None	-

Note: RVA, Rotavirus A; ETH, Ethiopia; BD, Bahir Dar; WT, wild type; GE, genomic equivalent; C_T, threshold cycle; NoV, norovirus; HAdV, human adenovirus and HPeV, human parechovirus

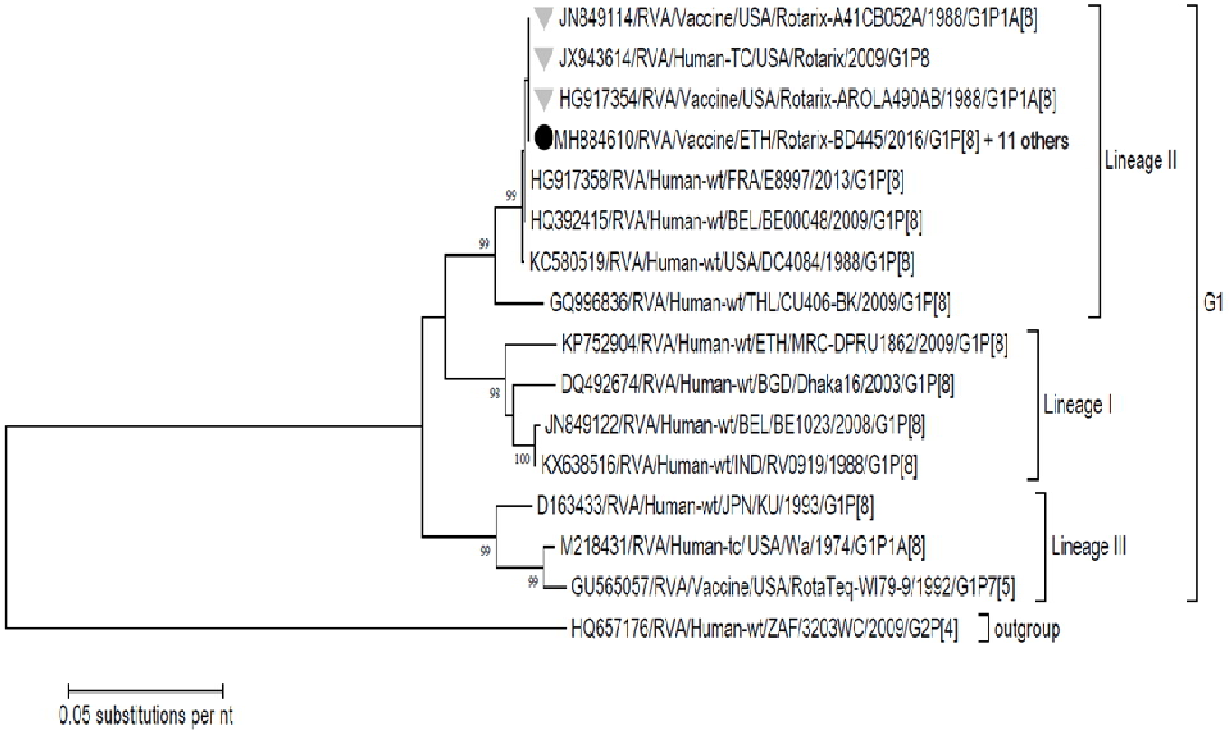

Figure 1*: Phylogenetic trees are based on nucleotide sequences of the VP7 gene segments rotavirus A strains. Isolates of the present strains are marked with a filled circle and other Rotarix vaccine strains are labeled by a lled triangle. The tree was constructed using the Neighbour-Joining method implemented in MEGA7 software. Bootstraps values (1000 replicates) above 75% are shown.*

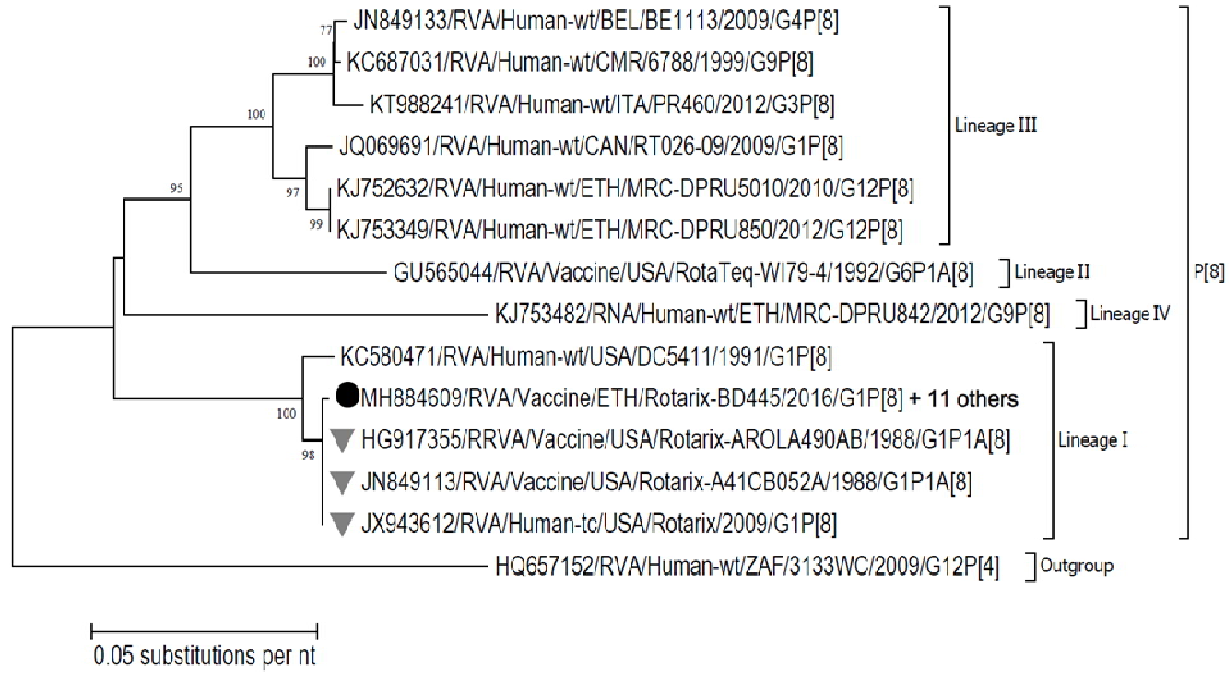

Figure 2: *Phylogenetic trees are based on nucleotide sequences of the VP4 gene segments rotavirus A strains. Sequences of the present vaccine viruses are marked with a filled circle and other Rotarix sequences are labeled by a lled triangle. The tree was constructed using the Neighbour-Joining method implemented in MEGA7 software. Bootstraps values (1000 replicates) above 75% are shown.*

DISCUSSION

The current study presents the detection of Rotarix vaccine virus from fecal samples of diarrheic children primarily collected for the purpose of rotavirus surveillance. Rotarix vaccine virus was identified in 2.7% of the children attending outpatient health institutions because of acute gastroenteritis.

In many African countries, rotavirus infection and associated hospitalization have significantly decreased following the introduction of the vaccine (6). Unlike these observations, recent post-vaccine rotavirus surveillance in Ethiopia failed to show a significant decrease in diarrhea-related hospital admissions. The possible reasons for this could be recent introduction of the vaccine, low vaccination coverage, lack of routine surveillance system and appropriate diagnostic tools (8, 13).

In the present study, vaccine virus was detected in seven children older than 1 year up to 28 months. Similarly, previous studies also reported that vaccine strains were detected in vaccinated children following vaccination (15, 16). The detection of the vaccine strains in children who are not candidates for vaccination indicates probable horizontal transmission and risk of associated symptomatic infections has been shown repeatedly (15,16,17). An association between rotavirus vaccine strains and symptomatic infections has been reported (15).Ina previous population study, the cutoff point threshold cycle of 26.7 was correlated with symptomatic infection attributable to rotavirus(20). In this study, a threshold cycle less than this cutoff point was observed in some of the fecal samples, which supports the likely occurrence of symptomatic infections. On the other hand, horizontal transmission of vaccine strains could increase herd immunity to rotavirus disease (14). Hence, the present study provides additional information for the growing shreds of evidence of possible benefits and risks of rotavirus vaccination.

Limitation of the study: the vaccination status of the children, their history of close contact with vaccinated children and clinical manifestations were not recorded. Besides, further investigations for parasitic and bacterial infections were not performed. Hence, the real contribution of the vaccine virus for the observed gastroenteritis in this study was not clear.

In conclusion, this study highlights the detection of Rotarix vaccine virus in under-5 children with an unknown history of vaccination. The detection of rotavirus vaccine strains in older children suggests horizontal transmission is very likely. High viral copies in some of the children indicate the possibility of vaccine-related symptomatic infections. Further studies are necessary to understand the transmission dynamics and risk of vaccine derived strains in childhood rotavirus infections.

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Statement of Ethics: The study was approved by the ethical review boards of the University of Gondar. Written informed consent was obtained from parents or guardians.

Disclosure statement: The author declare no conflicts of interest.

Author contributions: The author designed the study, carried out the laboratory assay, and wrote the manuscript.

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