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Prevalence of Group a Rotavirus before and after Vaccine Introduction in Mukuru Informal Settlement in Kenya

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Abstract

Background: Rotavirus vaccines have been shown to be a lifesaving and cost-effective public health intervention in Africa and have resulted in reduced rotavirus mortality. In Kenya, rotavirus diarrhea causes 19% of hospitalizations and 16% of clinic visits among children <5 years of age and causes 4471 deaths and 8,781 hospitalizations per year. Nationally, rotavirus disease costs the health care system $10.8 million annually. It is estimated that routine vaccination with a 2-dose rotavirus vaccination series would avert approximately 2,467 deaths (55%), 5,724 hospitalizations (65%), 852, 589 clinic visits (59%) and would save 58 disability-adjusted life-years (DALYs) per 1000 children annually. In July 2014, Kenya introduced rotavirus vaccine into its routine expanded programme immunisation, with two doses given at 6 and 10ths week of age.WHO recommend having surveillance studies before and after vaccine as baseline data and monitoring the possible effect after vaccine introductions. The aim of this study was to determine the prevalence of rotavirus in pre- and post-vaccine stool samples collected from children under five years, attending two selected clinics in Mukuru informal settlement in Nairobi, Kenya.

Methods: Archived samples collected during a *Salmonella* surveillance study (SSC No. 2074) conducted between July 2013 and July 2015 were used for this study. A total of 270 samples (150 pre-vaccine and 120 post-vaccine) were tested for rotavirus using ELISA Prospect kit (Oxoid Ltd UK) and data analyzed using SPSS version 20.

Results: Rotavirus prevalence was 10% (15/150) and 5% (6/120) in pre-vaccine and post-vaccine samples respectively. There was significant difference in prevalence pre and post vaccine samples for children less than 12 months (P=0.014), 13-24 months (P=0.002) and over 49 months (P=0.01). However, there was no difference in prevalence for age categories 25-36 and 37- 48 months.

Conclusion: This study showed a reduction in prevalence of Group A rotavirus in Mukuru selected clinics one year after vaccine introduction into National immunization program in Kenya. Rotavirus prevalence differed significantly for cases less than 12 months, 13-24 months and over 49 months pre and post vaccine introduction. However, there was no difference in prevalence for age category 25-36 and 37- 48 months thus the vaccine proved to have a significant protection in the most vulnerable group of children.

Keywords: Rotavirus, Kenya, vaccine, pre-vaccine, post-vaccine, prevalence, Kenya.

Introduction

Background information

Rotavirus is a leading cause of diarrhea in children under five years (WHO 2009). It is estimated to cause 527,000 deaths globally with approximately 232,000 occurring in South Asia and Sub-Saharan Africa (Mwenda *et al.*, 2010).

Previous studies in Kenya (Nyanza and Western Kenya) found that rotavirus infections caused 19% of hospitalizations and 16% of clinic visits for diarrhea among children <5 years. Between 2005-2007 the annual mortality burden associated with rotavirus was estimated to be 68 deaths per 100,000 children (Tate *et al.*, 2009). A prospective surveillance for children <13 years at Kilifi County Hospital reported admissions with diarrhea as

3,296 (22%) of which 2,039 were tested for rotavirus with 588 (29%) positive cases reported (Nokes *et al.*, 2008).

Rotavirus is contracted through the fecal oral route. It is highly contagious and spreads easily from person to person through contaminated hands and objects following contact with fecal matter (WHO, 2012). Children with severe rotavirus infections have frequent diarrhea and vomiting leading to dehydration and often need to be rehydrated with intravenous fluids, Oral rehydration solution (ORS) and zinc tablets (WHO, 2009). In developing countries, this type of emergency care is largely inaccessible, making the rotavirus prevention through vaccination critical to saving children's lives (Albert *et al*., 2012). Other measures that would be combined in preventing diarrhea include improvement of water quality, hygiene and prevention of bacterial and parasitic infections (Navaneethan and Giannella, 2008).

Following clinical trials in America and Europe that showed high efficacy of over 85-98% against severe rotavirus infection, there was recommendation of introduction of rotavirus vaccine trials in countries in Africa and Asia (WHO 2007). Rotavirus vaccine studies in South Africa and Malawi reported efficacy estimates ranging between 57-64% against a hospital admissions end point for rotavirus associated diarrhea and similarly reduced the prevalence of rotavirus in young children (Madhi *et al.*, 2010, Msimang *et al*., 2013). Similar studies in Mali and Ghana have reported an efficacy of 83% in the first year of life (Armah *et al.*, 2010, Christabel *et al*., 2014). In Kenya, a rotavirus vaccine trial in the Western part of the country reported efficacy of 63.9% through two years of follow up in 1308 infants (Felkin *et al.*, 2012).

WHO extended the recommendation for introduction of rotavirus vaccine into national immunization programs in African countries (WHO 2009, Jiang *et al*., 2010, Armah *et al*., 2010) following review of clinical trial data from Africa. Through Global Alliance for Vaccines and Immunization (GAVI), 19 countries in Africa have introduced the vaccine in their immunization programs (www.gavi.org). Rotarix (GSK) was included in Kenya immunization program in July 2014 in all government health facilities. Children aged below one year were administered two doses vaccine at 6 and 10 weeks of age (www.gavi.org/country/kenya).

WHO, however, also recommends local disease surveillance studies prior to the introduction of vaccines. Part of this should be monitoring of rotavirus diarrhea cases and as well as circulating strains. Having baseline surveillance for prevalence of rotavirus positive cases and trends pre and post vaccine introduction is important in monitoring the effects of the vaccine and for the country to get an understanding of epidemiology of rotavirus (WHO /IVB/08/16). This study set out to determine the possible influence of vaccination on number of rotavirus positive after vaccine introduction in Kenya.

Methodology

Ethical statement

Ethical approval for the study was obtained from the Scientific Ethics & Review Unit (SERU) KEMRI/SERU/CMR/P0026/3144. This study was part of the ongoing *Salmonella* surveillance study (SSC 2074). Consent from parents/guardians for their children had already been sought by personnel involved in study SSC 2074. All samples were anonymized and no personally identifiable information was present on the same labels.

Study population and sample collection

This study was conducted in two outpatient health facilities, Medical Missionaries of Mary and Reuben Medical center in Mukuru urban informal settlement situated 15 km East of Nairobi city. This is an overcrowded area with closely clustered houses, garbage dumps, open drains and inadequate sanitation.

A single time point fecal sample was collected in sterile specimen collecting tubes (poly pots). Upon receipt, the samples were transported in cool boxes for other bacterial pathogens testing at the Centre for Microbiology Research, Kenya Medical Research Institute in Nairobi. An aliquot of sufficient sample was stored at -20^{0}C until use in this study.

Inclusion criteria were children below 5years of age, with diarrhea 3 or more watery, non-bloody stool within 24 hour period.

Exclusion criteria was, <5years, bloody stool, no stool available or stored sample insufficient.

A total of 270 stool samples were purposively selected from 3995 children that had been recruited between July 2013-July 2015. Additional demographic data including age, sex and clinical characteristics were also gathered from case report form.

Laboratory Procedures

ELISA(Enzyme Linked Immunoassay) using Prospect^{TM} rotavirus kit

Diagnosis of rotavirus was based on the detection of virus antigen in human stool using a commercial kit **(**PROSPECT^{TM} Oxoid LMD, UK). This is a qualitative technique which utilizes a polyclonal antibody in a solid phase sandwich enzyme immunoassay to detect group specific antigen present in Group A rotaviruses.

Briefly, 1ml of sample diluent (Tris Buffered Saline solution) was added to approximately 0.1g of solid fecal material (small pea-sized portion) or approximately 100ul of liquid stool and mixed thoroughly on a vortex mixer and left to settle for 10 minutes prior to testing.

To conduct the ELISA test, 100ul of prepared sample was added in each well of the microtitre plates supplied with the kit that is pre coated with antibody. A 100µl horse peroxidase-conjugated anti-human antibody was added to each well and mixed by rotating the plate manually 3 times. The plates were then incubated at 20-30^{o}C for 60 minutes and subsequently washed 4 times using 350-450 µl PBS in each well. Substrate 100 µl was then added to each well and incubated at 20-30^{o}C for 10 minutes, followed by addition of 100µl of the 0.46 mol/L sulphuric stop solution. The plates were mixed thoroughly and read at 450nm within 30 minutes of addition of the stop solution using BioTek Synergy4-Gen5 microplate reader. The negative control value was set at less than 0.150 absorbance units while positive control was a value greater than 0.500 absorbance units. The cut-off value was calculated by adding 0.200 absorbance units to the negative control value. Any specimen with absorbance value less than the cut-off was negative while those above the cut off were considered positive.

Statistical Analysis

Data analysis was carried out using the SPSS version 20. Chi square tests were used to compare proportions of rotavirus cases before and after vaccine and to determine significance at 95% CI and P<0.05. To assess the possible influence of vaccination, the data was divided into two: pre vaccine (July 2013-July 2014) and post vaccine (July 2013-July 2015).

Results

In the study period from July 2013-July 2015, 270 samples (150 before vaccine and 120 after vaccine) were used and demographic data regarding age and gender was recorded. Out of the 150 children for the period before vaccine, there was equal distribution of sample size 75 (50%) of female and male. For the period after vaccine introduction 56 (46.7%) were female while 64 (53.3%) were male. There was no significant (P>0.05) difference between males and females in the selected sample set (Table 1).

Table 1: Sample size distribution by gender in Mukuru Informal settlement pre and post vaccine introduction.

Gender	Female		Male		Total				
	N	%	N	%	N	%	Chi square	df	P-value
Pre-vaccine	75	50	75	50	150	55.6	0	1	1
Post-vaccine	56	46.7	64	53.3	120	44.4	0.533	1	0.465
Total	131	100	139	100	270	100			

Age wise distribution of patients showed significant difference (P=0.001) in sample distribution among the age categories for pre vaccine period (Table 2).With the highest age category being 13-24 months 24/150 (28%). However, the sample sizes for post vaccine were equally distributed (P>0.05) in the different age categories.

Table 2: Age stratification of sample size distribution in Mukuru Informal settlement pre and post vaccine introduction

Age range (months)	<12		13-24		25-36		37-48		>49		Total			
	N	%	N	%	N	%	N	%	N	%	N	Chi square	df	P-value
Pre-vaccine	36	24	42	28	39	26	23	15.3	10	6.7	150	23.667	4	0.001
Post-vaccine	18	15	18	15	33	27.5	26	21.7	25	20.8	120	6.583	4	0.160
Total	54	20	60	22.2	72	26.7	49	18.1	35	13	270			

Rotavirus was more prevalent before vaccine introduction in children <12 months 6/36 (16.7%) and 13-24 months 4/42 (9.5%) (Table 3). In children 25-36 months and 37- 48 months, however, there was no difference in rotavirus prevalence pre and post vaccine introduction.

Table 3: Age stratification of rotavirus positives in Mukuru Informal settlement pre and post vaccine introduction

Vaccine	Pre-vaccine			Post vaccine					
	Negative	Positive	Total	Negative	Positive	Total			
	N %	N %	N %	N %	N %	N %	chi-square	df	P-value
Age(Months) <12	30(83.3)	6 (16.7)	36(100)	18(100)	0(0)	18(100)	6	1	0.014
13-24	38(90.5)	4(9.5)	42(100)	16(88.9)	2(11.1)	18(100)	9.6	1	0.002
25-36	35(89.7)	4(10.3)	39(100)	31(93.9)	2(6.1)	33(100)	0.5	1	0.485
37-48	22(95.7)	1(4.3)	23(100)	25(96.2)	1(3.8)	26(100)	0.184	1	0.668
>49	10(100)	0.0(100)	10(100)	24(96)	1(4)	25(100)	6.429	1	0.011

Rotavirus was detected slightly higher in female 8/75 (10.7%) and 7/75 (9.3%) in male for the period before vaccine. For the period after vaccine introduction cases in male were slightly higher 4/64 (6.3%) compared to female 2/56 (3.6%).

Table 4: Rotavirus positives distribution by gender in Mukuru Informal settlement pre and post vaccine introduction

Vaccine	Pre-vaccine			Post vaccine					
	Negative	Positive	Total	Negative	Positive	Total			
	N %	N %	N %	N %	N %	N %	chi-square	df	P-value
Female	67(89.3)	8 (10.7)	75(100)	54(96.4)	2(3.6)	56(100)	2.756	1	0.097
Male	68(90.7)	7(9.3)	75(100)	60(93.8)	4(6.3)	64(100)	0.871	1	0.351

A total of 15/150 (15%) and 6 /120 (5%) cases tested positive for rotavirus pre and post vaccine introduction periods respectively. The figure below shows the overall prevalence between pre and post vaccine. Pre vaccine was ($p<0.05$) higher prevalence compared to post vaccine.

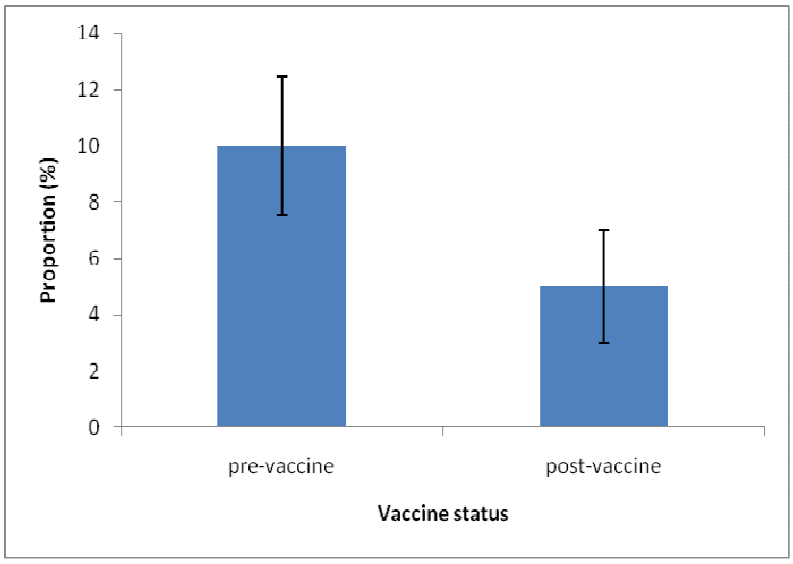

Figure1: Prevalence of rotavirus infection by vaccine group (pre vaccine and post vaccine)

Discussion

Rotavirus is among the major causes of diarrhea leading to death in children in developing countries. Data on prevalence of rotavirus in Kenyan informal settlements before and after vaccine introduction is limited. In this study, we set out to compare prevalence of rotavirus infections one year before and one year after rotavirus vaccine introduction in outpatient facilities.

We found rotavirus prevalence of 10% (15/150) and 5% (6/120) during the pre-vaccine and post-vaccine periods, respectively, which was statistically confirmed (P=0.05) leading to a 50% reduction of rotavirus cases. A previous study carried out in Mukuru for the period before vaccine (2012-2013) reported a rotavirus prevalence of 23% in children (Raini *et al*., 2015) that is almost double the prevalence that we observed in our study. Analysis of the prevalence confirms findings from a previous study done in the same location three years ago before vaccine introduction to be 24%, indicating that rotavirus was still a major pathogen causing diarrhea in children in Kenya (Gikonyo *et al*., 2010). The high prevalence of rotavirus in the current and other studies in East Africa reflect the high burden of rotavirus reported elsewhere in sub-Sahara Africa (Nakawesi *et al*., 2010, Mwenda *et al*., 2010, Sabrina *et al*., 2007).

Rotavirus prevalence differed significantly for cases less than 12 months, 13-24 months (p value-and over 49 months pre and post vaccine introduction. However, there was no difference in prevalence for age category 25-36 and 37- 48 months. There was no significant difference in prevalence between male and female study participants over the two periods. Reported prevalence from Mukuru study post vaccine introduction on age categories compared well to a study in Rwanda, with decrease in proportion of rotavirus positive after introduction of pentavalent rotavirus vaccine in May 2012, with the greatest effect in children directly protected by vaccine 13-24 months in 2014-2015 (Ngabo *et al.*, 2016). However, same study reported a reduction in admission by 61-70% including older children age-ineligible for vaccination suggesting an indirect protection through reduced transmission of rotavirus.

Although the effectiveness of rotavirus vaccine in Rwanda is promising, the effectiveness is still less than that in developed countries. Finland, for instance, with a high vaccine coverage, the effect of rotavirus vaccination has seen at 88% reduction of admittances to hospital for rotavirus gastroenteritis, with most of the remaining cases occurring in older children too old to be vaccinated in the programme (Hemming *et al*., 2013).

Generally, information on rotavirus vaccine effectiveness from Rwanda and other African countries is non-existent and future studies should address the effectiveness of vaccines after complete and uncomplete series of vaccinations. Indirect protection of children too old to have been vaccinated with rotavirus vaccine has been previously reported in several high-income and middle-income countries including the USA and El Salvador (Lambert *et al*., 2009, Payne *et al*., 2011). Comparative analysis of the assessment of vaccine effectiveness after the introduction of rotavirus vaccine into routine immunization programme in South Africa and Malawi showed that the vaccine was 57-64% effective against hospital admissions for rotavirus and reduced prevalence in young children (Groome *et al.*, 2014, Msimang *et al.*, 2013). Partially similar data was reported in a study conducted in

Brazil, where prevalence rates dropped from 11.12% in the pre vaccine years 2002-2006 to 5.07% in the post vaccine period 2007-2011 (Andressa et al., (2013).

Conclusion

This study reported that rotavirus infection majorly affects children aged 12 to 24 months who were mostly eligible to be vaccinated. Prevalence of rotavirus decreased substantially after rotavirus vaccine implementation. The introduction of vaccine in the National immunization program in Kenya can be effective measure to decrease the rotavirus disease in young children and reduce the financial burden to the Kenyan health system. These data support the use of rotavirus vaccine in Kenya and highlight the benefits of routine vaccination against rotavirus in low-income settings. However with only one year of both pre and post vaccine data from Mukuru informal settlement, it is too early to establish the trends of rotavirus cases. In order to assess the impact of the vaccine on the disease occurrence, continuous monitoring of rotavirus cases is needed. The use of sentinel surveillance sites in Kenya from July 2014 will also provide the most comprehensive information after vaccine introduction. Research on other enteric viruses will provide evidence of indirect protection from rotavirus vaccination in a high-burden, low income settings. Routine use of rotavirus vaccine could have a large impact on diarrhea-associated mortality and morbidity in Kenya.

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Conflict of interest

The authors declare no conflicts of interest.

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