Original Article

ROTAVIRUS GASTROENTERITIS SURVEILLANCE AND PREVALENCE ASSESSMENT AMONG UNDER FIVE CHILDREN IN RWANDA

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ABSTRACT

Rotavirus remains the most common cause of severe childhood diarrhea worldwide and of diarrheal mortality in developing countries. Despite the efforts made by the government of Rwanda and the stakeholders to reduce children mortality, the prevalence of rotavirus among under five children in Rwanda remains to be determined.

We conducted a hospital-based cross-sectional study that aimed at determining the prevalence of rotavirus infection in under five children presenting with gastroenteritis in eight hospitals in Rwanda. From June 2013 and August 2014 we collected and tested stool samples for the presence of rotavirus using an enzyme immunoassay and a Real Time-Polymerase Chain Reaction for genotyping. In 969 stool samples, 232 (23.94%) were rotavirus positive. Among them, 119 were genotyped. The highest prevalence was observed in July (4.3% and 5.1%) while the lowest one was observed in March (0.00%). Muhima Hospital had the highest prevalence (33.33%) whereas Kabgayi and Rwamagana Hospitals had the lowest (15.62 and 18.18%, respectively). Male children were more affected than females (25.8 versus 21.5%). We found that the prevalence was higher (31.10%) in children aged between 12 and 24 months than in other age groups. For genotyping, G9 [P8] was the most prevalent genotype as G9 prevalence was 54.6% whereas [P8] prevalence was 73.9%.

In conclusion, the prevalence of rotavirus gastroenteritis was high among children aged less than 5 years, and it was different according to age groups and among different hospitals.

Keywords: Rotavirus – prevalence – gastroenteritis – genotyping

RESUME

Rotavirus reste la cause la plus fréquente de diarrhée sévère de l'enfance dans le monde et de mortalité par diarrhée dans les pays en développement. Malgré les efforts déployés par le gouvernement du Rwanda et les partenaires pour réduire la mortalité infantile, la prévalence de gastro-entérite à rotavirus au Rwanda reste à déterminer.

Nous avons mené une étude transversale en milieu hospitalier qui visait à déterminer la prévalence de l'infection à rotavirus chez les enfants de moins de cinq présentant une gastro-entérite dans huit hôpitaux au Rwanda. De Juin 2013 à Août 2014, nous avons prélevé et examiné des échantillons de selles pour recherche de rotavirus en utilisant la technique d'immuno-enzymatique et la PCR en temps réel pour le génotypage. Dans 969 échantillons de selles collectés, 232 (23,94%) étaient positifs à rotavirus. Parmi eux, 119 ont été génotypés. La prévalence la plus élevée a été observée en Juillet (4,3% et 5,1%), tandis que la plus basse a été observée en Mars (0,00%). L'Hôpital de Muhima montrait une prévalence élevée (33,33%), tandis que les hôpitaux de Kabgayi et Rwamagana avaient la plus faible (15,62 et 18,18%, respectivement). Les enfants de sexe masculin étaient plus touchés que ceux de sexe féminin (25,8 contre 21,5%). Nous avons constaté que la prévalence était plus élevée (31,10%) chez les enfants âgés entre 12 et 24 mois. Pour le génotypage, G9 [P8] était le génotype le plus répandu dont la prévalence était de 54,6%, tandis que celle du [P8] était de 73,9%.

En conclusion, la prévalence de la gastro-entérite à rotavirus était élevée chez les enfants âgés de moins de 5 ans, et il était différent selon les groupes d'âge et entre les différents hôpitaux au Rwanda.

Mots-clés : rotavirus – prévalence – gastroentérite- génotypage

INTRODUCTION

Gastroenteritis is a catch all term for infection or irritation of the digestive tract, particularly the stomach and intestine; it is frequently referred to as the stomach or intestinal flu. Major symptoms include nausea and vomiting diarrhea, and abdominal cramps. These symptoms are sometimes accompanied by fever and overall weakness. Gastroenteritis typically lasts about three days. Adults usually recover without problem but children, the elderly, and anyone with an underlying disease are more vulnerable to complications such as dehydration [1].

The most common cause of gastroenteritis is viral infection. Viruses such as rotavirus, adenovirus, astrovirus, and calicivirus and small round-structured viruses (SRSVs) are found all over the world. Exposure typically occurs through the fecal-oral route, such as by consuming foods contaminated by fecal material related to poor sanitation. However, the infective dose can be very low (approximately 100 virus particles), so other routes of transmission are quite probable [2].

Typically, children are more vulnerable to rotaviruses. Annually, worldwide, rotaviruses are estimated to cause 800,000 deaths in children below age five. For this reason, much research has gone into developing a vaccine to protect children from this virus. Adults can be infected with rotaviruses, but these infections typically have minimal or no symptoms.

The greatest danger presented by gastroenteritis is dehydration. The loss of fluids through diarrhea and vomiting can upset the body's electrolyte balance, leading to potentially life-threatening problems such as heart abnormalities (arrhythmia). The risk of dehydration increases as symptoms are prolonged. Dehydration should be suspected when a child expresses dry mouth,

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increased or excessive thirst, or scanty urination is experienced. Symptoms of great concern include a high fever (102°F[38.9°C] or above), blood or mucus in the diarrhea, blood in the vomit, and severe abdominal pain or swelling. These symptoms require paying a special prompt medical attention [3].

METHODS

We collected stool samples during the acute phase of the infection, which is the period in which diarrhea is prevalent. Samples were collected from 6 district hospitals of Muhima (in central), Kibagabaga (in central), Rwamagana (eastern province), Kabgayi (southern province), Ruhengeri (northern and oustern province) and kabutare (southern province). These district hospitals were selected according to their geographical locations and also to the high prevalence of gastroenteritis [4]. In addition, two referral teaching hospitals including Kigali University Teaching Hospital (CHUK) and Butare University Teaching Hospital (CHUB) were included as main sites for study implementation.

After collection, we then put the samples into clean leak-proof containers then we took them to the laboratory for evaluation. Containers were identified with the particular enrollment codes with each sample having its unique identifier.

We stored the samples between 2 and 8°C in a refrigerator and transported them in a triple packaging system with ice packs and sent to CHUK laboratory for analysis. For genotyping, we sent the rotavirus positive stool samples to the Medunsa University Virology Laboratory, in South Africa.

For sample preparation, 1 ml we added to a suitable labelled container and we prepared a 10% suspension or dilution of fecal specimen by addition of approximately 0.1 g of solid feces (small pea sized portion) or approximately 100µl of liquid feces using transfer pipette in container for later use. We diluted specimens in ProspecT Rotavirus Sample diluents and we stored them at 2-8°C for up to 8days prior to testing. Weproceeded to open the foil pouch; remove the required number of microplate strips and place into microplate strip holder; used one well for negative control and one well for the Positive Control. We added 2 drops (or 100µl) of each diluted specimen, Negative Control or Positive Control should be included in each batch of tests. After addition of all specimens and controls, we added 2 drops (or 100 µl) of Conjugate to each microwell. We covered the plate and incubated the microwells at 20-30°C for 60 ± 5 minutes. We shake out or aspirated the contents of the wells. We washed by completely filling each well with diluted wash buffer (350-400 µl per well). We shake out or aspirated all fluid from wells after each wash. We washed a total of 5 times. After the last wash we removed contents and stroked plates on clean paper towels or aspirate. If using an automated washer, this should be programmed to complete 5 wash cycles. Washers must be correctly calibrated to ensure complete filling and emptying of microwells between each wash. After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of wash buffer. We added 2 drops (or 100µl) of substrate to each microwell, overed the plate and incubated the microwells at 20-30°C for 10 minutes. We stopped the substrate reaction by adding 2 drops (or 100µl) of stop solution to each microwell. We ensured thorough mixing of the microwells before reading the results. The coloured product is stable for to 30 minutes after addition of stop solution. Finally we red spectrophotometrically at 450 or 620 nm within 30 minutes of addition of the Stop Solution. Ensure the bottoms of the microwells are clean before reading. The reader should be blanked on air before the plate is scanned. If the spectrophotometer allows for the use of a reference wavelength (at 620 to 650 nm), dual wavelength reading should be performed. Calculate the cut off value by adding 0.200 absorbance units to the negative Control value, or mean value when more than one Negative control is included. Positive: clinical sample absorbance value > the cut off value. Negative: clinical sample absorbance value < the cut off value. Equivocal: clinical sample absorbance value within 0.010 absorbance units of the cut off value.

RESULTS

1.1 Distribution of population according to period

During 15 months 969 samples were collected from June 2013 to August 2014 then they were analyzed in the laboratory. We observed that 232 children were rotavirus positive. The test results are shown in table 1.

Table 1: Distribution of population according to period

Months	Negatives	Positives	Prevalence	Total
June 2013	42	30	41,7 %	72
July 2013	61	42	40,8 %	103
Aug 2013	47	38	44,7 %	85
Sept 2013	85	24	22,0 %	109
Oct 2013	121	5	4,0 %	126
Nov 2013	72	12	14,3 %	84
Dec 2013	45	4	8,2 %	49
Jan 2014	31	2	6,1 %	33
Feb 2014	35	1	2,8 %	36
Mar 2014	27	0	0,0 %	27
Apr 2014	23	4	14,8 %	27
May 2014	37	8	17,8 %	45
Jun 2014	27	12	30,8 %	39
July 2014	82	50	37,9 %	132
Aug 2014	2	0	0,0 %	2
Total	**737**	**232**	**23,9 %**	**969**

Prevalence of rotavirus infection among under five children from June 2013 to August 2014. In a total of 969 stool specimens 232 (23.9%) were rotavirus positive. The overall prevalence was 23.9%.

The highest prevalence in all tested samples was observed in July (4.3% and 5.1%) while the lowest one was observed in March (0.00%).

1.2 Prevalence of rotavirus according to hospitals

In the eight hospitals in which we conducted the study, the prevalence of rotavirus gastroenteritis in children less than 5 years of age was established. We noticed that in all hospitals rotavirus prevalence was different.

Table 2: Prevalence of rotavirus gastroenteritis in different hospitals.

Hospitals	Totals	Positives	Prevalence
CHUK	41	10	24.39%
CHUB	70	16	22.85%
Ruhengeri	110	23	20.90%
Kibagabaga	132	34	25.75%
Rwamagana	176	32	18.18%
Muhima	159	53	33.33%
Kabgayi	160	25	15.62%
Kabutare	121	39	32.23%
Totals	**969**	**232**	**23.94%**

The prevalence of rotavirus gastroenteritis was 33.33% at Muhima hospital; 32.23% at Kabutare; 25.75% at Kibagabaga; 24.39% at CHUK; 22.85% at CHUB; 20.90% at Ruhengeri; 18.18% at Rwamagana and 15.62% at Kabgayi.
The following figure shows the prevalence of rotavirus gastroenteritis among under five children in the eight hospitals namely CHUK, CHUB, Ruhengeri, Kibagabaga, Rwamagana, Muhima, Kabgayi and Kabutare.

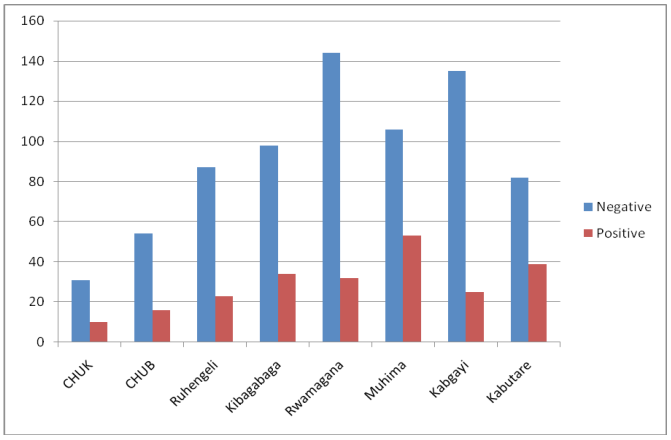


Figure 1: Prevalence of rotavirus gastroenteritis among under five children from different hospitals

The highest prevalence was observed in children attending Muhima Hopsital (33.33%) whereas the lowest was observed in those attending Kabgayi and Rwamagana Hospitals (15.62% and 18.18% respectively).
1.3 Prevalence of rotavirus according to sex
The prevalence of rotavirus gastroenteritis among children aged less than 5 years according to sex in the eight hospitals was determined. Males were more affected than females (25.8% versus 21.5%).

Table 3: Distribution of rotavirus among under five children according to the sex groups

Sex groups	Totals	Positives	Prevalences
Males	554	143	25.8%
Females	415	89	21.5%
Totals	969	232	23.94%

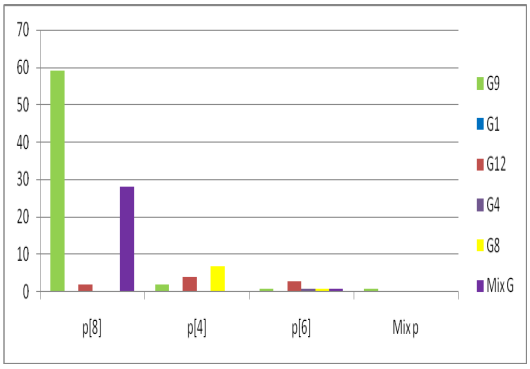
1.4. Prevalence of rotavirus according to age groups
In 969 samples that were collected in children aged from 0 to 60 months, four age groups were established and the prevalence was determined in each age group.

Table 4: Distribution of rotavirus gastroenteritis according to age groups

Age groups	Totals	Positives	Prevalences
0-5 months	55	4	7.27%
6-11 months	381	76	19.94%
12-24 months	418	130	31.10%
25-60 months	115	22	19.13%

Prevalence of rotavirus gastroenteritis according to age groups. Here we found that rotavirus gastroenteritis prevalence was higher (31.10%) in children aged between 12 and 24 months than in other age groups. The lowest prevalence (7.27%) was observed in infants aged from 0 to 5 months
1.5 Distribution of rotavirus genotypes in positive samples
We found out that the rotavirus genotypes present in tested samples were G (4,8,9, 12 and mixte G) and P (4,6,8 and mixte P).

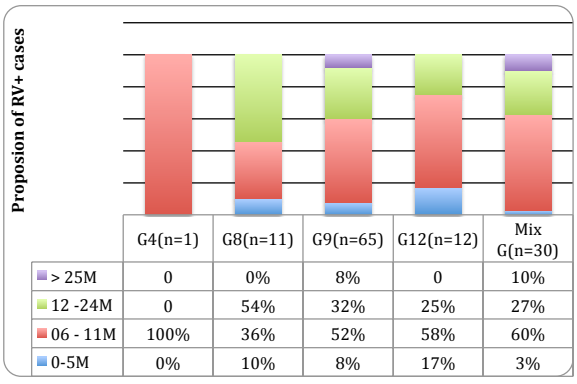
Figure 2: Genotype distribution among 119 rotavirus positive samples



This figure shows the distribution of genotype among 119 samples. Different genotypes including G4 =1(0.84%), G8=11(9.24%), G9 =65(54.62%), G12 =12(10.08%), and Mix G=30(25.21%) were identified. We found that the most prevalent genotype was G9=65(54.62%), whereas the least was G4 =1(0.84%),

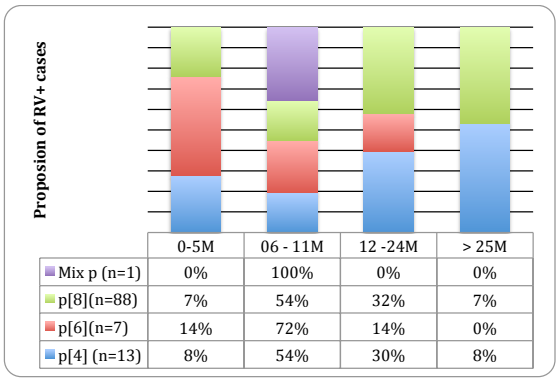
1.6. Distribution of different genotypes according to age groups
The distribution of rotavirus genotypes according to age groups was determined. G9 was distributed throughout all age groups, whereas the age group of 6 to 11 months was affected by all genotypes found during this research.

Figure 3: Distribution of G genotypes according to age groups



Data from this figure show that G[4]=1(100%) was found in the age group of 6 to 11 months; while G8 =11 was found in three age groups: 0-5months (10%); 6-11 months (36%); and 12-24 months(54%). G9 = 65 was distributed througout all age groups: 0.5 months (8%); 6-11 months (52%); 12-24months 32%); and >25 months (8%).

Figure 6: Distribution of genotype P according to age groups



	0-5M	06 - 11M	12 -24M	> 25M
Mix p (n=1)	0%	100%	0%	0%
p[8](n=88)	7%	54%	32%	7%
p[6](n=7)	14%	72%	14%	0%
p[4] (n=13)	8%	54%	30%	8%

About genotype P distribution in the age groups, the main findings were the following: in the age group of 0-5 months, P6 was the most prevalent (14%), Mix P was not found in this age group; while Mix P was found only in the age group of 6-11 months (100%). The P[8] was mainly found in this age group also (54%). It is in this age group that we found a mixture of all P genotypes. The age group of 12-24 months had three genotypes: P[8] (32%), P[4] (30%) and P[6] (14%). The last age group of >25 months had two genotypes: P[8] (7%) and P[4] (8%).

DISCUSSION

Our result findings showed that out of 969 patient samples, 232 (23.94%) were positive. These findings were similar to the ones from the study carried out in Kenya, where 4991 hospitalizations of children <5 years of age and 1134 (23%) were for acute gastroenteritis and stool specimens were obtained from 790 (70%). Rotavirus was detected in 211 (27%) specimens [5].

Among the 232 rotavirus positive children, 143 (25.8%) were males and 89 (21.5%) were females. The highest prevalence was observed in July. This could be due to the dry season where there is no rainfall with subsequent decrease of water supply and poor hygiene and sanitation. This can be compared to the findings from an study carried about rotavirus infection in the tropics by L. Kerren et al. [6] where on the basis of the evidence, they concluded that rotavirus responds to changes in climate in the tropics, with the highest number of infections found at the colder and drier times of the year. This is partially in accordance with our findings where the highest prevalence was observed in July but this is not so evident in April. The association of rotavirus infection and seasonal variations remains to be investigated deeply.

According to the findings, the highest prevalence (33.33%) was observed in children attending Muhima Hospital located in the capital Kigali City, while the lowest (15.62) was observed in Kabgayi Hospital located outside the capital. This high prevalence found in Kigali City could be attributed to the fact that many childcare workers and parents do not wash hands after changing diapers or before feeding the babies with food items. Challenging urban living conditions whereby the mothers have not enough time for breastfeeding their babies may also be one of the factors.

In fact, many mothers leave their children at home when they go to work and they prefer the artificial milk, fruits and bread as the diet for their children when they find themselves unable to satisfy their breast feeding needs during the working times; these food items may be the source of infection. The lowest prevalence observed at Kabgayi and Rwamagana could be the result of the living conditions according to which most the mothers live in rural area thus they are more likely to have enough time for breastfeeding and caring for their babies.

This can be compared to the findings from Nigeria which state that Playing with toys, attending day care, distance of source of water from toilet, eating of food not requiring cooking and playing with other children may serve as predisposing factors of rotavirus disease in these children. Children become infected if they put their finger in their mouth after touching something such as toys, books, clothing etc., that has been contaminated by stool of an infected person, this usually happen when children forget to wash their hands after using the toilet or before eating. Health care and childcare workers also spread the disease if they don't wash their hands after changing diapers. Rotavirus may also be transmitted through intake of faecally contaminated water or food, which usually occurs when, infected food handlers who prepare, salad, sandwiches, carrots and other foods that requires no cooking can spread the disease [7].

The prevalence of rotavirus gastroenteritis according to sex is surprising as males are more affected than females (25.8% vs 21.5%). These findings are similar to the findings from the study conducted in Morocco by Benhafid et al, in 2009 [8], where boys predominated among enrolled patients, compared with girls (63% vs 37% of hospital admissions; similar findings were from Nigeria where rotavirus were detected in faeces of 22(13.8%) children with acute diarrhea. Males excreted rotavirus at a significant higher rate than females a total of 64(40%) male and 96(60%) female cases of gastroenteritis were examined in this study. Rotavirus excretion in male cases was 14(8.8%) and female cases 8(5.0%) [7].

The most sticking finding is the prevalence of rotavirus infection which is significantly higher (31.10%) in children aged between 12 and 24 months, then it becomes lower (7. 27%) in infants of 0-5months of age. These findings can be compared to the findings from the study conducted in Maua Meru North District, Kenya where most patients with rotavirus infection were of the age of 3 - 60 months, with 79% being less than 18 months old [9].

The highest prevalence was observed in children aged between 1 and 2 years. This increase starts with the age of 6 months where we found the prevalence of 19.9%.

The raise in prevalence could be directly associated to the weaning age in which the children are not only fed by breast milk but also food items that might be infected by the virus are introduced to their diet. Furthermore those children are able to move and consume almost everything they accede without the awareness of the existence of pathogens.

Additionally, at this age, the immune system is less active against rotaviruses if they have not been immunized. These findings are in accordance with the findings from a study conducted from June 2006 through December 2008, where rotavirus surveillance was established. Of 5461 stool samples collected from children enrolled in 8 African countries, 2200 (40%) were positive for rotavirus. Ninety percent of all rotavirus hospitalizations occurred among children aged 3–12 months. These preliminary results indicate that rotavirus is a major cause of severe diarrheal disease in African children [10].

Regarding genotype distribution, G9 = 65(54.62%) was the most prevalent genotype (Figure 4) with (G9[P4] and G9[P8] as genotypes found all over the age groups as displayed in figures 5 and 6, while the least prevalent was G[4]=1(0.84%). This could be attributed to the fact that G[9] should be the most virulent genotype which affects more deeply the age group of 6-11 months (52%) as shown in Figure 6. Another surprising finding is that the age group of 6-11 months was vulnerable of all G and P genotypes with G[4] and Mix P found in that age group only. The underlying reason behind these findings should be attributed to the fact that this is the age of crawling children, which corresponds to the age when breastfeeding is reduced and it is supplemented by food. So children start eating food items whether given by parents and guardians, or picked up on their own from the contaminated ground and swallowed. Of all 119 genotyped samples, we found that G9 [P8] was the most prevalent. Unfortunately, the underlying cause remains to be investigated.

Our findings are in accordance with the ones from the study conducted in Uganda, where rotavirus was detected in 1844 (32.8%) of 5627 children with acute diarrhea that had stool specimens collected, and 93% of positive cases of rotavirus gastroenteritis were between 3 and 23 months of age, with highest prevalence in children 6–11 months of age. Rotavirus infections occurred throughout the year. During the surveillance period (2006–2012), a total of 354 positive stool samples were subjected to reverse transcription polymerase chain reaction and genotyping assays. The most common genotypes detected were G1P[8] (16.1%) and G9P[8] (15.3%) [11]. To sum up, the overall prevalence of rotavirus in under five Rwandan children was very high (23.94%) with a peak in the age group of 12 -24 months (31.10%) and in male children (28.8%). In Rwandan District Hospitals where the study was conducted, Muhima Hospital showed the highest prevalence (33.33%). July was the month that showed the highest prevalence. These are among the key evidences that rotavirus gastroenteritis is a threat to child health in Rwanda.

CONCLUSION

In conclusion, the aim of this cross sectional study conducted in eight Rwandan hospitals was to evaluate the prevalence of rotavirus gastroenteritis in selected hospitals according to sex and age groups among under five children attending hospital for medical care.

Our findings showed that rotavirus gastroenteritis was high among children aged less than 5 years, and it was different according to age groups. The prevalence of rotavirus infection was different among different hospitals and periods. G9 [P8] was the most prevalent genotype. Rotavirus remains the leading cause of severe viral gastroenteritis and our results recommend much attention should be taken for children aged less than 5 years.

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