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EPIDEMIOLOGICAL AND CLINICAL STUDY OF ROTAVIRUS DIARRHEAIN CHILDREN UNDER 5 YEARS AT PANZI GENERAL REFERENCE HOSPITAL (DRC / SUD-KIVU / BUKAVU)

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ABSTRACT: *Introduction:* Acute diarrhea is one of the leading causes of mortality and morbidity in children under 5 years old. Globally, it is responsible for around 4 million hospitalizations and 1.1 million deaths per year. Various etiological agents are implicated, including the viruses which constitute the 1st cause, mainly the rotavirus of group A.
The prevalence of rotavirus diarrhea is estimated at 38% globally, 34% in Africa and 62.7% in DRC. The objective of this study is to determine the hospital prevalence of rotavirus diarrhea in children under 5 years old, and to identify circulating genotypes.
Methodology: This is a cross-sectional descriptive study, carried out over 6 months in the pediatric emergency department at the HGR / Panzi. 68 fresh stool samples were collected and stored at the medical school research laboratory. Detection of rotavirus was achieved through the use of the enzyme-linked immunosorbent assay ELISA technique and genotype determination by RT-PCR.
Results: The prevalence of rotavirus gastroenteritis was 42.6%; Its frequency was highest in children under 24 months, who were the most affected, of which the 0-6 month group represented 37.9%, followed by that of 7-12 months (31%) and 13-24 months (27.6%).The associated factors were the age of our patients, the environment of origin, the level of education of the mothers, the feeding method of children ($p < 0.05$). The clinical signs associated with diarrhea were vomiting (34.48%) and fever (31.03%); The G1 genotype represented 21%, followed by G2 (7%) and G3 (3%); two P genotypes have been identified P [8] and P [4].
Conclusion: Rotavirus remains an important cause of acute diarrhea in children under 5 in our region as well as in the Democratic Republic of Congo. The introduction of the vaccine into the national immunization program will reduce its frequency somewhat.

KEYWORDS: rotavirus, prevalence, genotypes, children under 5, HGR / Panzi.

1 INTRODUCTION

Acute diarrhea is a leading cause of morbidity and mortality in children under 5, and is responsible for around 4 million hospitalizations and 1.1 million deaths per year [1, 2]. It is the second leading cause of death worldwide after pneumonia [3].

Diarrhea is most often caused by bacteria, parasites and viruses; the latter largely dominate the epidemiology of diarrhea, particularly through rotavirus. It is considered to be the main cause of acute diarrhea in infants and young children [4] mainly through rotavirus A [5].

It is a virus of the Reoviridae family, non-enveloped with a genome of 11 double-stranded RNA strands and a 3-layered capsid with 2 surface proteins: VP7 (glycoprotein G) and VP4 (protein sensitive to protease P); there are currently 27 G genotypes and 35 P genotypes [6]

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Rotavirus causes approximately 453,000 deaths each year, most of them in developing countries on the Asian continent, Africa and Latin America [4]. In developing countries, rotavirus infections account for 15 to 50% of gastroenteritis [7]. Their prevalence is estimated at 22% in America, 29% in Europe and 34% in Africa [8]. In the DRC, according to surveillance data at sentinel sites in Kinshasa [9] and Lubumbashi [10], the prevalence of acute gastroenteritis due to rotavirus is estimated at 62.7%; the main genotypes identified were G1P (8), G2P (4) and G2P (6).

The introduction of the rotavirus vaccine into the DRC's vaccination program is a further step in the prevention and reduction of cases of rotavirus diarrhea. The aim of this study is to determine the hospital prevalence, in the pre-vaccination era, and the genotypes of rotaviruses associated with gastroenteritis in children under 5 admitted to the pediatric emergency department of the General Reference Hospital of Panzi. This will allow us to assess the efficacy of the vaccine in subsequent studies.

2 MATERIAL AND METHODS

This is a cross-sectional descriptive study carried out from August 1, 2018 to January 31, 2019, lasting 6 months, in the pediatric emergency department at the HGR / panzi. Stool samples were taken from all children under 5 admitted for acute gastroenteritis within the first 2 days of hospitalization.

The stool was collected in a sterile jar and then sent directly to the Evangelical University in Africa laboratory, located a 5-minute walk from the hospital. They were stored at -20 ° C until use. A data collection sheet was established and completed after the approval of the parents or the caregiver. Parameters studied: age, sex, origin, feeding method of the child, level of education of mothers.

ANTIGEN DETECTION

Detection was performed by enzyme-linked immunosorbent assay ELISA (Oxoid Prospect rotavirus Kit) according to the manufacturer's instructions.

EXTRACTION OF VIRAL RNA

ANTIGEN DETECTION

The antigen was determined by the ELISA immunosorbent assay. (Oxoid Prospect rotavirus Kit) according to the manufacturer's instructions.

MOLECULAR TYPING

1. RNA extraction

RNA extraction was performed with the QIAamp DSP Virus Kit according to the manufacturer's instructions for 25 seropositive samples (QIAGEN GmbH, 40724 Hilden, GERMANY) at the microbiology laboratory at school of medicine of the Evangelical University in Africa (UEA). Quantification and evaluation of the quality of the extracted RNA was then carried out by electrophoresis with 1.5% Agarose Gel.

2. Conversion of RNA into complementary DNA

The RNA extract was converted into complementary DNA (cDNA) by the FIREScript_RTcDNA Synthesis kit (Solis BioDyne) using the FIREScript reverse transcriptase (200 U / µl), RiboGripRNase inhibitor (40 U / µl), the 10x RT Reaction Buffer with DTT 500 mMTris-HCl pH 8.3, 500 mMKCl, 30 mM MgCl2, 100 mM DTT, Oligo (dT) primers (100 µM) and random primers (100 µM), dNTP MIX (20 mM each) and nuclease-free water (Sterile water).

3. Reverse transcriptase amplification (RT-PCR)

Reverse transcriptase chain polymerization (RT-PCR) amplification was performed as described by Coulson et al. (11), Argüelles et al. (12) and Sadiq et al. (13). All reactions were performed with the Rotavirus vaccine (rotateq) as the positive control and nuclease-free water as the negative control. DNA amplification was done by conventional PCR in the thermo cycler at the Molecular Biology Laboratory of Evangelical University in Africa.

4. Gel electrophoresis and visualization

The RT-PCR products were separated by electrophoresis on a 1.5% agarose gel in TAE buffer consisting of Tris base, acetic acid and EDTA (SERVA Electrophoresis, Heidelberg, Germany) stained with nucleic acid. GelRed (Phenix Research Products, Candler, and USA) and PEC green in the microbiology laboratory of the Pan African University (Kenya / Nairobi)

STATISTICAL DATA ANALYSIS

For the data analysis, we coded and encoded in Microsoft Excel, and we analyzed them with XLSTAT 2016 software and Epi info version 3.5.1. Differences in proportions were tested using Fisher's corrected Chi-square test, a p value <0.05 was considered significant. The logistic regression method allowed us to calculate the ORs and their 95% confidence intervals.

ETHICAL CONSIDERATIONS

The protocol of our study received the approval of the National Committee of Ethics and Health (CNES 001 / DPSK / 129PM / 2018).

3 RESULTS

A total of 68 children under 5 were admitted for an acute gastroenteritis; after analysis of the samples, 29 returned positive for rotavirus, a proportion of 42.6%. Male children were the most infected with rotavirus (58.6%) compared to female subjects (41.4%) with a statistically significant difference (p <0.05), boys being 2.25 times more likely to have rotavirus diarrhea than girls (OR = 2.25; 95% CI; 1.14- 4.44)

Rotavirus infection was more marked in children under 24 months, the 0-6 month age group, which was the most affected (37.9%), followed by that of 7-12 months (31 %) and 13-24 months (27.6%). Bivariate analysis showed a statistically significant difference (p = 0.0000016); Children younger than 24 months were 6 times more likely to have rotavirus diarrhea compared to those older than 24 months (OR = 6.12; 95% CI; 2, 3-5.54).

Our study found that children who were exclusively breast-fed were less infected (20.69%) compared to those who were mixed undernourished (79.31%); the analysis showed a statistically significant difference (p <0.05). Children of educated mothers were more represented (86.21%) compared to those of less or uneducated mothers (13.79%); with a statistically significant difference (p <0.05). These children are 6.25 times more likely to have rotavirus diarrhea compared to those of less educated mothers (OR = 6.25; 95% CI; 2.18 - 17.96). The predominant G genotypes are G1 (21%), G2 (7%) and G3 (3%); the P genotype found is P [8] (10%) and P [4] (4%).

Table 1. distribution of the children according to studied parameters

Parameters		Rotavirus + (N=29)		Rotavirus - (N=39)		Total (N=68)		OR	IC to 95%		P-been worth
		N	%	N	%	N	%		Inf.	Sup.	
Age	<24 months	27	93,1	31	79,5	58	85,3	15,5	3,71	64,77	0,0000016
	> 24 months	2	6,9	8	20,5	10	14,7				
Sex	Masculine	17	58,6	27	69,2	44	64,7	2,25	1,14	4,44	0,0163
	Female	12	41,4	12	30,8	24	35,3				
Source	City	25	86,2	25	64,1	50	73,5	6,25	2,18	17,96	0,000097
	Except city	4	13,8	14	35,9	18	26,5				
Food	Mixed feeding and family dish	23	79,3	33	84,6	56	82,4	5,5	2,3	13,13	0,0000165
	Exclusive maternal food	6	20,7	6	15,4	12	17,6				
Educational level	Educated	25	86,2	25	64,1	50	73,5	6,25	2,18	17,96	0,0000972
	Not educated	4	13,8	14	35,9	18	26,5				

4 DISCUSSION

During our study, we registered 68 children under 5 with acute diarrhea; after analysis, the prevalence of rotavirus diarrhea was estimated at 42.6%.

Etiological studies on gastroenteritis in infants and children have shown that rotavirus is responsible for 40-50% of acute diarrhea in developing countries as well as developed countries [14]. A study carried out in 20 African countries is not far from our result, estimated the prevalence of diarrhea due to rotaviruses at 40% [15].

Our results are comparable to those found by other authors [17, 18, 19, 20], but remain inferior to the results found in the studies by Sangaji MK [10], Kabwe JP (20) and Pukuta [9], carried out in the other provinces of the DRC. This low prevalence can be explained by the fact that the period of our study was short, so it affected the size of our sample. Male children were more affected than female children, with a statistically significant difference.

Several other studies have shown more marked damage in males compared to females, Pol SS. in India (21) and Muendo C. in Kenya [22] However, other authors have found a predominance of women [10, 16, 22, 23], without there being a statistically significant difference.

According to Fischer, the predilection of the male sex could be explained by genetic and immunological factors [25]; men are more likely to develop a severe form of diarrhea, requiring hospitalization although both sexes are infected at the same rate [10, 25]. Children under 24 months of age have been most affected by rotavirus diarrhea; Studies have shown that the older you get, the less rotavirus diarrhea you get, because not only do you acquire immunity but you can also have asymptomatic forms [16].

Similar results were found by Tagbo BN [26] and Iyoha O. [27] in Nigeria; Pol SS. et al in India [21] and Jin-Tu Lou in China [28]; Children fed exclusively breast milk were less infected compared to those fed mixed feed;

Numerous studies have shown the protective role of breast milk against rotavirus infections in children under 6 months (29), however others have shown that breastfeeding offers protection only against severe forms of rotavirus diarrhea. [30]. Naficy et al. found a lower incidence of rotavirus diarrhea in infants fed exclusively with breast milk [31], while for Muendo et al., infants who were exclusively breastfed were about 1.5 times more likely to develop rotavirus diarrhea, although this was not statistically significant [22].

Compared to those of less educated mothers, with a statistically significant difference ($p = 0.0000972$), and have a risk of developing rotavirus diarrhea, 6.25 times more than the others. (OR = 6.25; 95% CI, 1.01-12). In a study on the prevalence of rotavirus diarrhea in Uganda [32], it was shown that children of mothers with an educational attainment (secondary or above) were twice as likely to have rotavirus diarrhea; contrary to the results found by Dennehy et al. in the United States, where children of less educated mothers were more likely to have rotavirus diarrhea (OR = 1.5; 95% CI, 1.0-2.3). [33]; This could be explained by the fact that children of educated mothers consult more compared to other children; but also most of the women work and thus leave the children on artificial milk, of which one does not control the preparation of the bottle well.

Our study allowed us to identify the G1, G2 and G3 genotypes represented respectively in 21%, 7% and 3% as well as the P8 and P4 genotypes found in 10% and 4% respectively. In Africa, several diversity of genotypes have been demonstrated in different regions. The G1 genotype is the most predominant and widely distributed in all regions, followed by types G9, G2 and G3; the P [8] genotype is the most common, followed by the P [4] and P [6] type (34)

In the DRC, several studies have demonstrated the predominance of the G1 and G2, P genotype [8] (9, 35, 36) but also with an emergence of the P genotype [6] Mixed forms have been found in Africa and the DRC, the most predominant of which is G1P [8] (8, 36). In our study we were unable to identify mixed forms. Despite the diversity of rotavirus strains, the monovalent vaccine, introduced in several African countries, has shown an efficacy of 59% (37).

5 CONCLUSION

Rotavirus remains a major cause of acute diarrhea in children under 5 in our region, as well as throughout the Democratic Republic of Congo. This diarrhea affects children 0-24 months more; and the genotypes found in our study have also been demonstrated in certain regions of the DRC. The recent introduction of the vaccine into the vaccination program in the DRC will make it possible to reduce somewhat the frequency of diarrhea linked to rotaviruses. Hence it would be necessary to carry out epidemiological and molecular surveillance in the post-vaccination period.

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