DIARRHEA BY ROTAVIRUS IN A REGIONAL PERUVIAN HOSPITAL: DETERMINATION OF CIRCULATING GENOTYPES.

Pablo Weilg Espejo[1†], Fiorella Orellana Peralta[1†], Hernán Cornejo Pacheres[2], Luis J. del Valle[3], Angela Cornejo Tapia [1], Jorge Bazán Mayra[2], Joaquim Ruiz[4], Juana del Valle Mendoza[1,5]*

[1]Faculty of Health Sciences. School of Medicine. Universidad Peruana de Ciencias Aplicadas - UPC, Lima, Peru. [2]Dirección Regional de Salud de Cajamarca (DIRESA-Cajamarca), Cajamarca, Peru. [3]Universidad Politécnica de Catalunya (UPC), Barcelona, Spain. [4]Barcelona Centre for International Health Research (CRESIB, Hospital Clínic - Universitat de Barcelona), Barcelona, Spain. [5]Instituto de Investigación Nutricional, Lima, Peru.

Abstract word count: 189

Text word count: 2421

† These authors contributed equally to this article.

*Corresponding author

Juana del Valle
Universidad Peruana de Ciencias Aplicadas
Av. San Marcos cuadra 2
Chorrillos
Lima
Peru

e-mail: jdelvall@upc.edu.pe
Phone. +51 1988040532
Fax. +51 1 3496023

ABSTRACT

Background: Gastroenteritis by rotavirus is responsible for approximately 810 annual deaths/year in children under 5 years in Peru and emerging rotavirus genotypes have led to concerns regarding cross-protection by the vaccines available. Moreover, there are no reports on the molecular-epidemiology of rotavirus diarrhea in Peru

Methodology: A total of 131 stool samples were obtained from children under 5 years old hospitalized from January 2010 to December 2012 in the Hospital Regional de Cajamarca, Peru. ELISA and RT-PCR techniques were performed for rotavirus detection. G and P typing of rotavirus-positive samples were obtained by semi-nested multiplex RT-PCR and sequencing was performed to confirm the PCR results.

Results: Of the 117 samples available, 18.80% (22/117) tested positive for rotavirus by ELISA and 35.90% (42/117) by RT-PCR. Among the G-genotype identified, G9 in 35.71% (15/42) and G12 in 33.33% (14/42) were the most prevalent. With the most common combination being G12/P6 in 23.81% (10/42).

Conclusions: A high prevalence of the G12/P6 genotype was detected. It is know that this genotype is not covered by the current vaccines available. More in depth studies are needed to know the current rotavirus genotypes presents in Peru.

Key words: Rotavirus, viral genotypes, epidemiology, acute gastroenteritis, Peru.

INTRODUCTION

Rotavirus is the most important cause of severe gastroenteritis accompanied by acute diarrhea in young children worldwide[1]. In Peru, human rotaviruses are responsible for approximately 810 annual deaths in children under 5 years old[2].

The genus Rotavirus belongs to the family of Reoviridae which contains 11 segments of double-stranded RNA that is located inside a triple-layered virus particle. Rotaviruses are classified into seven serogroups (A to G), based on the characteristics of the VP6 membrane protein and divided into G and P genotypes, according to the genetic and antigenic diversity of the two outer capsid proteins VP7 and VP4, respectively[3,4,5]. At least 27 G types and 35 P types have been identified and the most prevalent genotypes in humans are G1P [8], G2P [4], G3P [8], G4P [8], and G9P [8][6,7]. However, in the last few years Argentina, Chile and Brazil have reported new genotypes that have apparently spread in Latin America. Knowledge of their distribution, including detection of emerging genotypes, is crucial for rotavirus vaccination programs[8,9,10].

The major symptoms of rotavirus gastroenteritis in children include watery diarrhea, vomiting, respiratory symptoms, and fever[11,12]. Laboratory diagnosis of rotavirus infection is usually performed by antigen detection, using latex agglutination or more recently enzyme immunoassay (ELISA). However, in multiple studies both techniques have shown variable sensitivity and specificity with a risk of misdiagnosis[13,14]. Polymerase Chain Reaction (PCR) has become the preferred method for human rotaviruses detection as well as genotype characterization in epidemiologic studies[15,16].

In 2009, two rotavirus vaccines were introduced by the Peruvian National Immunization Program. A live attenuated vaccine called *Rotarix* specific for the G1P[8] genotype and it is administered in a 2-dose series in infants and children. The second vaccine is *Rotateq*, a

live oral pentavalent formula for serotypes G1, G2, G3, G4 and G6 [17]. Both vaccines have shown to be safe, providing more than 70% and 90% protection against any rotavirus diarrhea and severe diarrhea, respectively[18].

There are no studies on the current rotavirus genotypes circulating in Peru, and the emergence of new strains has led to new concerns regarding cross-protection by the present vaccine formulations. Hence continuous surveillance of rotaviruses for the investigation of the emergence of new strains is required, including the collection of baseline data aimed to evaluate the vaccines commercially available.

In this study we assessed the prevalence of rotavirus infection and genotypes using ELISA and PCR amplification of viral nucleic acid derived from stool specimens of children under 5 years old from the Regional Hospital of Cajamarca in Peru.

MATERIAL AND METHODS

Study population and collection of specimens

A total of 131 stool samples were obtained from children under 5 years old hospitalized with acute gastroenteritis from January 2010 to December 2012 in the *Hospital Regional de Cajamarca*, Peru. Samples were collected between 1 to 3 days after disease onset. Clinical data were registered by physicians in the hospital. Acute gastroenteritis was defined as the occurrence of diarrhea lasting less than 14 days along with symptoms such as vomiting, fever, dehydration, and abdominal pain according to the European Society of Paediatric Infectious Disease[19]. Fever was defined as an axillary temperature of ≥ 37.5 ºC. Nutritional status was based on weight-for-age Z scores (WAZ), calculated using the least mean square method and the 2000 CDC Growth Reference[20]. Dehydration status was established according to World Health Organization (WHO) criteria[21]. Fecal samples were transported at 4°C to the laboratory of the Microbiology Department of *Dirección Regional de Salud de Cajamarca*, Peru. The samples were stored at -20°C, until be sent to be processed in the Research Center for Health Sciences, *Universidad Peruana de Ciencias Aplicadas*, Lima, Peru.

This study was approved by the Ethics Committee of the *Universidad Peruana de Ciencias Aplicadas* and an informed consent was signed by the parents before sample analysis was performed.

Rotavirus detection by ELISA

Rotavirus antigen (group A rotavirus-specific VP6 proteins) was detected using an enzyme-linked immunosorbent assay (ELISA; RotaClone, Meridian Bioscience).

Extraction of dsRNA

Rotavirus-positive fecal suspensions were used for viral double-stranded RNA (dsRNA) extraction using the QIAamp viral RNA kit (Qiagen GmbH, Hilden, Germany) in accordance with the manufacturer's instructions. Viral dsRNA obtained after extraction was stored at -20°C until use.

Amplification of the Rotavirus VP4 and VP7 genes. Rotavirus dsRNA was reverse transcribed using the OneStep RT-PCR kit (Merck4Biosciences, Germany). After denaturation of the RNA at 80°C for 5 min, the RT-PCR comprised a reverse transcription step at 50°C for 30 min.

The primers used for amplification of VP7 gene sequences have been previously described by Gouvea et al[22], Griffin et al.[23], and VP4 gene sequences by Gentsch et al.[24]. The PCR mixture was 50 µl, distributed as follows: 25 µl of enzyme mix (Taq polymerase, 2.5 mM $MgCl_2$, 15 mM Tris / HCl pH 8.3, 50 mM KCl, 200 µM of each deoxynucleotide) (Kapa Biosytem, USA), 20 pmol of each primer (Macrogen, Seoul, Korea), and 5 µl of cDNA.

The PCR conditions were: 95ºC for 10 min, followed by 45 cycles of 94ºC for 30 sec52ºC for 45 sec and 72ºC for 1 min, with a final elongation of 10 min at 72 °C. The amplification products were analyzed by gel electrophoresis on 2% agarose (FMC, Rockland, ME) gel containing 3 mg/L ethidium bromide. Sequencing was performed to confirm the PCR results. Amplified products were recovered from the gel, purified (SpinPrep™ Gel DNA Kit, San Diego, USA) and sent for commercial sequencing service (Macrogen, Seoul, Korea).

G and P genotyping by semi-nested multiplex RT-PCR

Rotavirus dsRNA was reverse transcribed using the OneStep RT-PCR kit (Merck4Biosciences, Germany). G and P typing of the strains was performed by semi-nested multiplex PCR assays using consensus and type-specific primers [22,23,24].

The PCR conditions were: 95ºC for 10 min, followed by 45 cycles of 94ºC for 1 min, 52ºC for 1 min and 72ºC for 1 min, with a final elongation of 10 min at 72 °C. The amplified products were analyzed, recovered and sequence as mentioned above to confirm the PCR results.

Data analysis

Statistical significance was established using the Fisher exact test. The differences were considered as significant with a p-value<0.05. Statistical analyses were performed with SPSS software (Microsoft SPSS-PC+, v.15.0; SPSS, Chicago, IL, USA).

RESULTS

Study Population

Of the 131 samples collected from children under 5 years and hospitalized for acute gastroenteritis, 14 samples were excluded from the protocol analysis because they failed to meet the inclusion criteria. Of the remaining 117 samples, 22 (18.80%) tested positive for rotavirus by ELISA and 42 (35.90%) were positive for rotavirus gene amplification by RT-PCR (table 1), indicating the latter as a more suitable assay for rotavirus diagnosis.

Among all the positive rotavirus samples, a higher distribution of cases [45.25% (19/42)] was observed in children older than 18 months (table 2). Of all the rotavirus-positive cases, 35.71% (15/42) received mixed lactation, 30.95% (13/42) only formula, and 23.82% (10/42) exclusive breastfeeding. In addition, 26.19% (11/42) of the patients had some degree of malnutrition.

The most common symptoms of the patients with a positive rotavirus sample were fever in 64.29% (27/42) and vomiting in 57.15% (24/421). In this group mild diarrhea was observed in 42.86% (18/42), moderate diarrhea in 26.20% (11/42) and severe in 19.04% (8/42). On the other hand, one third of rotavirus cases presented mild to moderate dehydration and only 1 case of severe dehydration was observed. During hospitalization and using only clinical criteria, 42.86% (18/42) of patients received common antimicrobial drugs including ampicillin, amikacin, chloramphenicol and cephalexin (table 3).

Rotavirus detection and genotyping

ELISA rotavirus detection showed a sensitivity of 38.10% and a specificity of 92.00% using the RT-PCR as a reference standard; Also a positive and negative predictive values of

72.73% and 72.63% respectively were obtained with a 95% confidence interval (C.I.) (table 4).

The amplification of the VP7 and VP4 genes by a semi-nested multiplex RT-PCR showed the presence of the following G genotypes: G9 in 35.71% (15/42) and G12 in 33.33% (14/42) were the most prevalent, while G3 (15.00%), G4 (5.00 %) G1 (2.50%) and G2 (2.50%) were present to lesser frequencies. No G8 was detected. (Table 5).

Regarding VP4 amplification for the P genotypes, the most frequent strains were P6 (59.38%) and P8 (34.38%) while P4 was found in only 2 cases (6.25%). Complete rotavirus genotyping was achieved in 71.43% (30/42) of samples, G12/P6 being the most common genotype in 23.81% (10/42), followed by G9/P6 in 19.05% (8/42) and G9/P8 in 11.90% (5/42) of patients.

DISCUSSION

Rotavirus gastroenteritis is responsible for 40% of the hospitalizations of children worldwide[1]. In Peru, the Epidemiologic Surveillance report of 2008 showed that rotavirus was detected in 35.80% of all acute gastroenteritis in pediatric patients under 5 years old. Higher incidences were correlated with children older than 18 months while the most vulnerable population was found those 11 months old or younger [2]. In this study, prevalence of rotavirus was 18.80% (22/117) and 35.90% (42/117) using ELISA and RT-PCR respectively. Approximately, one third of all the children enrolled were between 18 months and 5 years of age and 45.25% (19/42) of them had had a rotavirus-positive test, consistent with previous studies.

Polymerase chain reaction is used widely for rotavirus detection and has become the most popular method for human rotavirus genotyping epidemiology studies[14,15,25]. Different studies have reported a sensitivity ranging from 84.8-99% and a specificity of 73.3-99.7% with ELISA applying different laboratory assays as reference standards (virus isolation, electronic microscopy, polyacrylamide gel electrophoresis)[13,27,29]. Using RT-PCR as a confirmatory test for rotavirus detection, we found that ELISA has a very low sensitivity (38.10%) while having a relatively high 92.00% specificity (92.00%).

Rotavirus gastroenteritis is characterized by watery diarrhea, which normally last about 5-6 days, associated with fever and vomiting in 50% and 75% of the cases, respectively[28]. In our series, fever was the most common symptom, present in 64.29% (27/42), followed by vomiting in 57.15% (24/42) of patients. On admission 42.86% (18/42) of all rotavirus-positive cases presented mild diarrhea and 45.24% (19/42) were found moderate to severe

diarrhea. Moreover, 42.86% (18/42) of the rotavirus cases received antibiotics on hospitalization using only clinical criteria.

Rotavirus genotype surveillance is critical for understanding the efficacy of vaccination, and different behaviors of dominant strains have been reported in the last 20 years in several Latin American countries[29]. As an example, during a 20-year surveillance period in Brazil, G1 was the most prevalent type during 1986, emerging again in 1993, 1998 and in 2003. G3 became the dominant strain during 1987-1992 and G9 peaked in 1999-2002 and 2005. Finally, G9 was the dominant type in 2006; however in 2007 an increase of G2, with both serotypes co-circulating at comparable proportions revealed that rotavirus types may concomitantly prevail in different geographical locations[30].

A recent meta-analysis has reported that in Latin America the most common G type detected is G1 (34.2%), followed by G9 (14.6%) and G2 (14.4%). In P types, clearly P8 was the most frequently detected (56.2%), followed by P4 (22.1%) and P1 (5.4%). Overall, the most prevalent G-P type associations were G1P8 (17.9%), G2P4 (9.1%) and G9P8 (8.8%) [29]. The same study reported that G1 has a prevalence of 61.3% in Peru based on a 12-article review[29]. However, none of these Peruvian studies were designed to determine rotavirus genotypes and only 2 articles from 1996 described the prevalence of G1, G2, G3 and G4 prevalence. Unfortunately, in Peru there is no rotavirus genotyping epidemiologic surveillance program and since 1996 no further data have been published. In our study, G9 in 35.71% (15/42) and G12 in 33.33% (14/42) were the most dominant rotavirus genotypes, with the most common combination being G12/P6 in 23.81% (10/42), followed by G9/P6 in 19.05% (8/42) and G9/P8 in 11.90% (5/42) of patients. This G9 dominance was observed in a similar study from Chile published in 2012, in which increases in G9 had

been reported in the last few years. However, in this study the predominant combination was G9P8 (76%) followed by G1P8 (6%) and no G12 was detected[10].

Genotyping of VP7 and VP4 could not be performed in 4.76% (2/42) and 23.81% (10/42) of the cases respectively. This is probably because these strands belong to other G and P proteins that have been reported in Latino America in the last few years, such as G5, G10, G11, P9 or other unidentified genes[10,29]. It is important to mention that we found a high prevalence of the G12 genotypes in our series, being a relatively new genotype in which vaccine efficacy is not fully understood[10,31].

In Latin America, the G9 rotavirus serotype has been associated with a significantly longer duration and higher frequency of diarrhea, longer duration of vomiting, increased hospitalization rate and more-severe dehydration[8]. Although this association remains under debate, we found that almost all our G9/P6 patients had moderate to severe diarrhea associated with fever and vomiting, with more severe diarrhea being reported in patients with G3/-, G9/P6, -/P6 and -/P4 (table 5). However, it is important to remark that the present study was not addressed to establish a genotype-clinical association.

Despite the G9/P8 and G9/P6 genotypes not being included in the two vaccine formulas, different studies have demonstrated a reduction in the number of severe cases (65-85%)[32,33]. This effect could be due to the presence of P6 and P8 antigens or because of a cross-reaction with different G genotypes or other structural antigens[10].

This study presented two limitations. The first involves the need to transport the samples from Cajamarca to Lima, despite to be frozen we can not ruled out possible sample missmanipulation during any part of the process. The second limitation is that it was

designed only for rotavirus detection in the patients stool specimens. Therefore, the presence of other confections can not be ruled out..

As in other Latin American countries, epidemiologic surveillance programs for rotavirus genotypes are necessary in Peru, especially related to children who could most benefit from these vaccines. This study demonstrated that prevailing rotavirus genotypes in children can dynamically change over time and highlights the need for further investigations to better establish the burden of rotavirus, the impact of the disease and how the efficacy of vaccination programs is affecting.

Authors' contributions: PW and FO conceived the study; PW, FO and JV designed the study protocol; JV was responsible for obtaining funding and laboratory work supervision; PW and FO performed the PCR for rotavirus detection and genotyping; HC carried out the immunoassays. AC was responsible for the clinical assessment, samples collection and database completion. PW, FO, LJ, JR and JV drafted the manuscript; LJ, JV and JR critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. PW and JV are guarantors of the paper.

No author has a conflict of interests.

Acknowledgements

We thanks Donna Pringle for the idiomatic correction.

Funding: This work was supported by the 1st Concurso Incentivo a la Investigación de la Universidad Peruana de Ciencias Aplicadas (UPC). Lima-Perú

JR has a fellowship from the program I3, of the ISCIII [grant number: CES11/012.

REFERENCES

1. World Health Organization (WHO). Estimated rotavirus deaths for children under 5 years of age 2006. [Internet]. Geneva: WHO. [Accessed September 7, 2012; Cited on September 19, 2013] Available at: http://www.who.int/immunization_monitoring/burden/rotavirus_estimates/en/index.html.
2. Luna Pineda, MA. Vigilancia epidemiológica de diarreas y vigilancia de rotavirus basada en sitios centinela 2008. [Internet]. Lima, Perú. Dirección General de Epidemiología (DGE). [Accessed September 12, 2012; Cited on September 19, 2013] Available at: http://www.sabin.org/files/miguelangel.co.pdf.
3. Jain V, Das B, Bhan M, et al. Great diversity of group A rotavirus strains and high prevalence of mixed rotavirus infections in India. J Clin Microbiol 2001; 39 : 3524-9.
4. Kasule M, Sebunya TK, Gashe BA, et al. Detection and characterization of human rotavirus among children with diarrhoea in Botswana. Trop Med Int Health 2003 ; 8: 1137-42.
5. Mota-Hernandez F, Calva JJ, Gutierrez-Camacho C, et al. Rotavirus diarrhea severity is related to the VP4 type in Mexican children. J Clin Microbiol 2003; 41: 3158-62.
6. Patton J. Rotavirus diversity and evolution in the post-vaccine world. Discov Med. 2012; 13:85-97.
7. .Gutierrez I, Steyer A, Boben J, et al. Sensitive detection of multiple Rotavirus genotypes with a single reverse transcription-Real-Time Quantitative PCR Assay. J Clin Microbiol 2008 ;46: 2547-54.

8. Linhares AC, Verstraeten T, Wolleswinkel-van den Bosch J, et al. Rotavirus serotype G9 is associated with more-severe disease in Latin America. Clin Infect Dis 2006 1;43: 312-4.
9. Nozawa C, Kentopf G, Da Silva E, et al. Detection and characterization of human rotavirus in hospitalized patients in the cities of Ponta Grossa, Londrina and Assai-Pr, Brazil. Infect Dis 2010; 14: 553-7.
10. Lucero Y, Mamani N, Cortés H, et al. Rotavirus genotypes in children with gastroenteritis assisted in two public hospitals from Chile: viral strains circulating in a country without a universal vaccination against rotavirus. Rev Chilena Infectol 2012; 29: 142-8.
11. Huppertz HI, Salman N, Giaquinto C. Risk factors for severe Rotavirus gastroenteritis. 2008; 27: S11-9.
12. Chen SY, Chang YC, Lee YS, et al. Molecular epidemiology and clinical manifestations of viral gastroenteritis in hospitalized pediatric patients in Northern Taiwan. J Clin Microbiol 2007; 45: 2054-7.
13. Al-Yousif Y, Anderson J, Chard-Bergstrom C, et al. Evaluation of a latex agglutination kit (VirogenRotatest) for detection of bovine rotavirus in fecal samples. Clin Diagn Lab Immunol 2001; 8: 496-8.
14. Eing BR, May G, Baumeister HG, et al. Evaluation of two enzyme immunoassays for detection of human rotaviruses in fecal specimens. J Clin Microbiol 2001; 39: 4532-4.
15. Noppornpanth S, Poovorawan Y. Comparison between RT-PCR and rapid agglutination test for diagnosis of human Rotavirus infection. 1999 Southeast Asian J Trop Med Public Health; 30: 707-9.

16. Pongsuwanna Y, Taniguchi K, Wakasugi F, et al. Distinct yearly change of serotype distribution of human rotavirus in Thailand as determined by ELISA and PCR. Epidemiol Infect 1993; 111: 407-12.
17. De Oliveira LH, Danovaro-Holliday MC, Matus CR, et al. Rotavirus vaccine introduction in the Americas: progress and lessons learned. Expert Rev. Vaccines 2008; 7: 345-53
18. Angel J, Franco M, Greenberg H. Rotavirus vaccines: recent developments and future considerations. Nat Rev Microbiol 2007; 5: 529-39
19. de Miguel Duran F, Perdomo Giraldi M. Gastroenteritis aguda. Deshidratación. Pediatr Integral 2011; 15: 54-60.
20. Centers for Disease Control and Prevention (CDC). 2000 CDC Growth charts. [Internet]. Atlanta: CDC. [Accessed January 3, 2013; Cited on December 19, 2013] Available at: http://www.cdc.gov/growthcharts
21. World Health Organization (WHO). Pocket book for hospital care of children: guidelines for the management of common illness with limited resources. . [Internet]. Geneva: WHO. [Accessed September 7, 2012; Cited on September 19, 2013] Available at: http://www.who.int/maternal_child_adolescent/documents/9241546700
22. Gouvea V, Glass RI, Woods P, et al. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol. 1990; 28: 276-82.
23. Gentsch JR, Glass RI, Woods P, et al. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol. 1992; **30:** 1365-73.

24. Griffin, DD, Nakagomi T, Hoshino Y, et al 2002. Characterization of nontypeable rotavirus strains from the United States: identification of a new rotavirus reassortant (P2A[6],G12) and rare P3[9] strains related to bovine rotaviruses. Virology 294: 256–69.
25. Pun SB, Nakagomi T, Sherchand JB, et al. Detection of G12 human rotaviruses in Nepal. Emerg Infect Dis.. 2007 ;13: 482-4.
26. Leland DS, Ginocchio CC. Role of cell culture for virus detection in the age of technology. Clin Microbiol Rev 2007; 20: 49-78.
27. Dennehy P, Gauntlett D, Tente W. Comparison of nine commercial immunoassays for the detection of rotavirus in fecal specimens. J Clin Microbiol 1988; 26: 1630-4
28. IkoHuppertz H, Salman N, Giaquinto C. Risk factors for severe rotavirus Gastroenteritis.2008; 27: S11-9.
29. Linhares A, Stupka J, Ciapponi A, et al. Burden and typing of rotavirus group A in Latin America and the Caribbean: systematic review and meta-analysis. Rev Med Virol 2011; 21: 89-109
30. Estes M, Kapikian A. Rotaviruses. In: Fields Virology, 5th ed, Vol. 2, Knipe DM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, (eds). Lippincott Williams & Wilkins: Philadelphia, PA, 2007; 1917–1974.
31. Steele A, Neuzil K, Cunliffe. Human rotavirus vaccine Rotarix™ provides protection against diverse circulating rotavirus strains in African infants: a randomized controlled trial. BMC Infect Dis. 2012; 12: 213

32. Ruiz-Palacios G, Pérez-Schael I, Velásquez R, et al. Safety and efficacy of an attenuated vaccine against rotavirus severe gastroenteritis. N Engl J Med 2006; 354: 11-22.
33. Vesikari T, Matson D, Denehi P, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006; 354: 23-33.

Table 1: Comparison of techniques used to identify rotavirus.

DIAGNOSTIC METHOD	ELISA		PCR	
	Frequency	**Prevalence (%)**	**Frequency**	**Prevalence (%)**
Positive	22	18.80	42	35.90
Negative	95	81.20	75	64.10
Total	117	100.00	117	100.00

Table 2: Age distribution of ≤ 5 year old children and those with rotavirus.

Age months	N (%) of Rotavirus positive	N (%) of Rotavirus negative	N (%) total patients
0 – 5	9 (21.43)	12 (16.00)	21 (17.95)
6 – 11	3 (07.14)	19 (25.33)	22 (18.80)
12 – 17	6 (14.28)	22 (29.34)	28 (23.93)
18 and above	19 (45.25)	19 (25.33)	38 (32.48)
Unknown	5 (11.90)	3 (04.00)	8 (06.84)
Total	**42 (100.00)**	**75 (100.00)**	**117 (100.00)**

Table 3: Characteristics of Rotavirus-Positive and Rotavirus-Negative cases.

Characteristics	Rotavirus Positive (N=42), n (%)	Rotavirus Negative (N=75), n (%)
Lactation		
▪ Exclusive breastfeeding	10 (23.82)	23(30.67)
▪ Formula	13 (30.95)	7 (09.33)
▪ Mixed	15 (35.71)	31 (41.33)
▪ Unknown	4 (09.52)	14 (18.67)
Symptoms before hospitalization		
▪ Vomiting	24 (57.15)	38 (50.67)
▪ Fever	27 (64.29)	34 (45.33)
▪ Malnutrition	11 (26.19)	8 (10.67)
Diarrhea		
▪ Mild (1-6)	18 (42.86)	23 (30.67)
▪ Moderate (7-10)	11 (26.20)	8 (10.67)
▪ Severe (≥ 11)	8 (19.04)	24 (32.00)
▪ Unknown	5 (11.90)	20 (26.66)
Degree of dehydration		
▪ No dehydration	27 (64.29)	50 (66.67)
▪ Mild or moderate	14 (33.33)	23 (30.67)
▪ Severe	1 (02.38)	2 (02.66)
Treatment received during hospitalization		
▪ Oral rehydration	15 (35.71)	45 (60.00)
▪ Intravenous rehydration therapy	9 (21.43)	8 (10.67)
▪ Antibiotic	18 (42.86)	22 (29.33)

Table 4: Sensitivity and Specificity of ELISA

		95 % C.I	
		Inferior Limit	**Superior Limit**
Disease Prevalence	**35.90%**	27.39%	45.35%
Correctly diagnose patients	**72.65%**	63.50%	80.29%
Sensitivity	**38.10%**	23.99%	54.35%
Specificity	**92.00%**	82.79%	96.71%
Positive Predictive Value	**72.73%**	49.56%	88.39%
Negative Predictive Value	**72.63%**	62.36%	81.04%

C.I. Confidence Interval

Table 5: Human Rotavirus genotype distribution by multiplex polymerase chain reaction.

G type / P type	Frequency (n=42)	Prevalence (%)	Diarrhea				Fever			Vomiting		
			L (17)	M (10)	S (4)	U (11)	Y (28)	N (8)	U (6)	Y (27)	N (9)	U (6)
G1 / ─	1	02.38	1				1			1		
G2 / ─	1	02.38				1			1			1
G3 / ─	6	14.29	2		1	3	3	2	1	4	1	1
G9 / ─	2	04.76	1			1	1		1	1		1
G3 /P6	1	02.38		1			1				1	
G4 /P8	2	04.76	1	1			2			1	1	
G9 /P6	8	19.05	1	4	1	2	6	1	1	6	1	1
G9 /P8	5	11.90	3	1		1	3	1	1	2	2	1
G12 / P6	10	23.81	6	2	1	1	7	2	1	8	1	1
─ / P4	2	04.76		1	1		1	1		1	1	
G12 / P8	4	09.52	2			2	3	1		3	1	

L= Mild, **M=** Moderate, **S=** Severe, **U=** Unknown, **Y**= YES, **N**= NO

4