Molecular epidemiology of Rotavirus diarrhea among children in Nepal: Emergence of G12 and G9 strains

Sherchand JB[1], Tandukar S[1], Sherchan JB[2], Gurung S[3], Dhakwa JR[4], Bichha RP[4], Mahaseth C[4]

[1]Tribhuvan University Institute of Medicine, Public Health Research Laboratory and Microbiology;

[2]Department of Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel;

[3]Program for Immunization Preventable Diseases (IPD), Kathmandu, Nepal;

[4]Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal

Correspondence address: Dr. Jeevan B. Sherchand

Tribhuvan University Institute of Medicine, Public Health Research Laboratory and Microbiology

Email: jeevanbsherchand@gmail.com

Abstract

Introduction: Rotavirus is the leading cause of diarrhea and dehydration among infants in both developed and developing countries. The primary objective of this study was to find the magnitude of rotavirus disease burden and genotypic variations of rotavirus.

Methodology: Questionnaires and stool samples were collected from 1003 enrolled children under 5 years of age attending tertiary care Children's Hospital with acute watery diarrhea during January to December 2012. Rotavirus in stool samples was detected by Enzyme Immuno Assay (EIA) and strains detected from rotavirus positive samples were genotyped by Reverse-Transcription Polymerase Chain reaction (RT-PCR).

Results: Among these, 356 (35.4%) cases were positive for rotavirus by EIA, among the positive cases, 344 samples underwent genotyping by RT-PCR. Rotavirus positive cases were predominant in children who were admitted to the hospital which was 37.8% (115 out of 336). Overall G12 was the most prevalent genotype (52.3%), followed by G1 (17.7%), G2 (10.17%) and G9 (8.1%). The P types identified were P[6] (55.23%), P[8] (20%), and P[4] (12.5%).

Conclusion: The study reveals that rotavirus gastroenteritis accounted for more than one-third of all cases of acute diarrhea. Use of rotavirus vaccines may reduce of high burden of rotavirus diarrhea in children. Emergence of G12 and G9 strains proves the immediate need of vaccine in Nepal.

Key words: Molecular epidemiology, Rotavirus, Nepal

Introduction

Human rotaviruses are classified into seven serogroups (A-G)[1,2]. Group A rotaviruses are largely recognized as the single most important known aetiological agents of acute gastroenteritis in both infants and young children worldwide[3,4]. Currently, 23 G genotypes and 32 P genotypes have been described (5). Out of them, 12 G and 15 P, the combinations of G1P8, G2P4, G3P8, and G4P8 are the most frequently found genotypes in humans[5,6,7,8].

Rotavirus belongs to the Reoviridaefamily and the virion is comprised of three concentric protein layers. The outer capsid consists of two proteins, VP7 and VP4 that are used to classify rotavirus strains into G (glycoprotein) and P (protease sensitive) genotypes respectively (9, 10). Group A rotaviruses are responsible for 2 million hospitalizations worldwide; in developing countries, these rotaviruses cause 4,00,000–5,00,000 deaths annually in children <5

years old[1,2,3].Rotaviruses belong to the Reoviridae, and their genome consists of 11 segments of double stranded RNA[4]. The genesegment coding for the VP7 glycoprotein is the basis forgenotyping group A rotaviruses into at least 15 G-genotypes.Among them, G1, G2, G3, G4 and G9 are the mostcommon G-types in humans. Theimportance of type-specific immunological protectionagainst rotavirus disease is still under discussion[5].

This study confirms the high disease burden of rotavirus gastroenteritis which might be reduced by the use of effective vaccines and also emergence of G12 and G9 genotype make challenge to generate alternate vaccination.

Methodology:

Sample collection and preparation: Human rotavirus strains were identified from stool samples of children withacute gastroenteritis who visited government tertiary care children's hospital, Kathmandu, Nepal between January–December 2012. All children were under 5 years and the samples were collected within two days of admission. Rotavirus in the stool samples were diagnosedby (Rotavirus Ag ELISA, Pro Spect, USA).

Clinical methods

Children who were brought to the pediatric emergency unit were screened for acute watery diarrhea by medical officers and nurses. The guardians/ caretakers of all enrolled children were explained about the study and informed consent was taken.

Study setting, sample and data collection

This cross sectional descriptive study was done using1003 stool samples from patients with acute watery diarrhea with fever, vomiting and abdominal pain. Stools collected were stored at −70°C before analysis was done using EIA and molecular methods. The data and clinical information were recorded on a standardized questionnaire and entered into EPI-Info analysis software.

Measurement variables

Data was collected using a pre-coded data collection tool. Information regarding age, sex, type of nutrition (breast-fed), hospitalization and clinical symptoms such as diarrhea, vomiting, fever and convulsion, were recorded for each child.

Rotavirus detection: The specimens were tested by a solid-phase sandwich-type enzyme immunoassay method (Rotavirus Ag ELISA, Pro Spect, USA).

Rotavirus Genotyping

All stool samples were prepared as 10% suspensions in phosphate-buffered saline (0.2 M; pH 7.4). The suspensions were centrifuged for 5 min at 1,600 g, and then 250µl of the supernatant was used for total-RNA isolation using Trizol reagent, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Purified RNA was resuspended in 30l of sterile nuclease-free water (Promega Corporation, Madison, WI) and stored at 70°C. When required, stool samples were diluted to the desired concentrations using healthy stool, Premier sample diluent buffer (Meridian Bioscience Inc., Cincinatti, OH), tap water, or environmental water, and afterwards, 250l was subjected to total-RNA isolation with Trizol reagent. In some cases, luciferase (luc) RNA (Promega, Madison, WI) was added (2 ng per sample) directly to the Trizol reagent before RNA extraction as an external control for the extraction procedure and to report potential PCR inhibitory effects inherent to the samples. A negative control for the extractionprocedure, consisting of buffer only, was always included.

Rotavirus G and P genotyping

The cDNA is used as a template for G- and P- typing PCRs. Five micro liters of cDNA is used in amplification reactions for the first round VP7 and VP4 gene products in 50 µl reactions and 1 µl of this amplified product serves as template for the 2nd round mµltiplex PCR. For VP7 genotyping, the first round PCR primers VP7-F and VP7-R will amplify an 881bp region of the VP7 gene. The nested mµltiplex PCR will incorporatethe reverse primer (VP7-R) and the primers specific for amplification of genotypes G1, G2, G3, G4, G8, G9, G10 and G12. Primers Con2 and Con3 will used in the first round PCR to amplify an 876bp fragment of the VP4 gene. The second round PCR will include the consensus primer Con3 and primers specific for genotypes P[4], P[6], P[8], P[9], P[10] and P[11]. The primers sequences, cycling conditions and amplicon sizes for VP7 and VP4 genotypes are used and were analyzed by RT-PCR as described earlier.[12, 39]

Data Interpretation

Gel Electrophoresis and Documentation: The genotypes were identified based on the PCR amplicon size on gel electrophoresis. PCR ampliconswere resolved in 2% agarose gels stained with ethidium bromide (0.5 mg/ml) in Tris – boric acid – EDTA (TBE) buffer at constant voltage. Images were photographed under UV light using a gel documentation system.

Analysis: All case based data and clinical information was recorded on a standardized questionnaire and entered into EPI-Info computer based analysis software. Differences in the proportions of each genotype were assessed by using the x2 test.

Ethics: The present study was approved by the Institutional Review Board of Institute of Medicine, Tribhuvan University, Kathmandu Nepal

Results

Epidemiology of Rotavirus

A total of 1003 cases were enrolled in oneyear (January-December 2012) period that fulfilled the enrollment criteria for the study. Out of them 356 (35.4%) cases were positive for rotavirus by EIA, among the positive cases, 344 samples underwent genotyping by RT-PCR. Rotavirus positive cases were predominant in children who were admitted to the hospital i.e. 37.8% (115 out of 336).

Clinical Presentation in hospitalized patients

On the basis of clinical features, rotavirus positive cases were found more among those having nausea 40.8% (259 out of 653) followed by vomiting 39.6 % (283 out of 733), abdominal pain 38.6% (283 out of 733) and fever 33.4% (186 out of 556).

Month wise distribution of rotavirus cases in hospitalized patients

The month wise distribution showed that rotavirus diarrhea cases occurred mostly from January to May and rotavirus positive percentage was found more from month of December (49.2%) to April (43.04%). In this study, highest percentage of rotavirus was seen in the month of January (58.8%), followed by February(54.5%) and March (51.1%) as shown in figure 1.

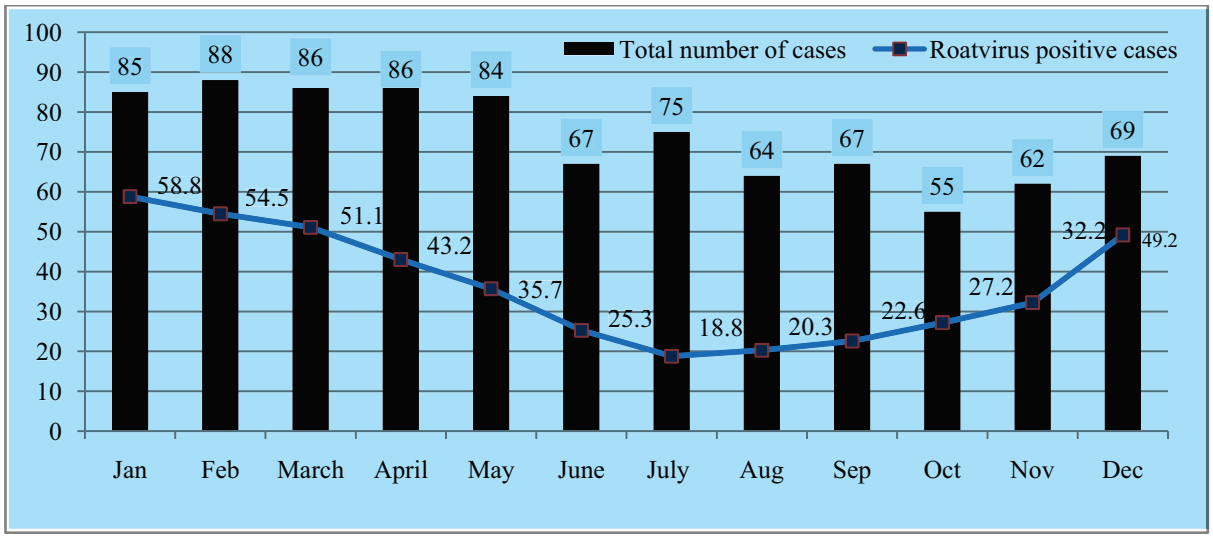

Figure 1: Month wise distribution of rotavirus infection.

Genotyping of ELISA positive rotavirus

The distribution of the G and P types are depicted in figure2 and 3. Four different G types were detected: G1, G2, G9 and G12. Of these, G12 was the most prevalent genotype (52.3%), followed by G1 (17.70%), G2 (10.17%) and G9 (8.1%) as depicted in figure 2. Three different P types were detected: P4, P6 and P8. The P types identified as shown in figure 3were P[6] (55.23%), P[8] (20%), and P[4] (12.5%).

The most common G-P combinations genotypes depicted in figure 4 identified were: G12P[6] (48%), G1P[8] (12.8%), G2P[4] (9%), G1P[6] (3%), G12P[8] (2.03%), G9P[6] (1.4%), G9P[4] and G1P[4] holding same (0.9%) and G2P[6] (0.3%). In this study mixed (14.5%), partially typed (4.4%) and untyped (3%) strains were also found.

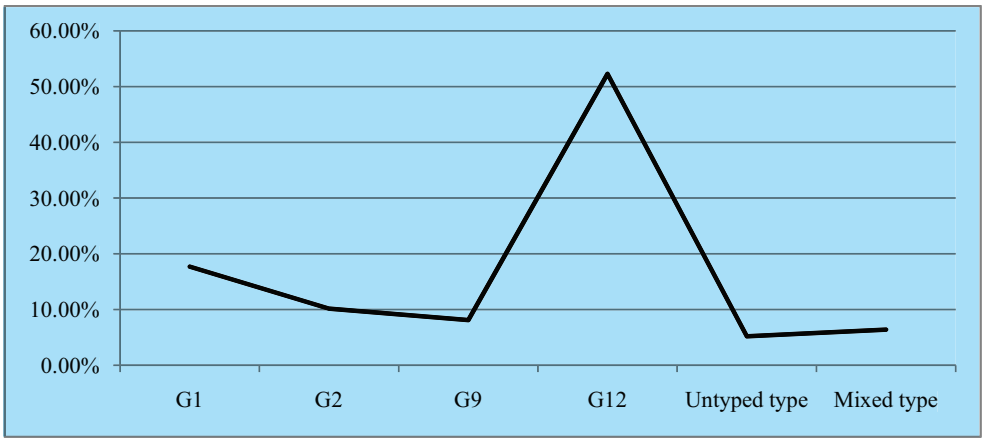

Figure 2: Distribution of G genotypes among rotavirus infected children

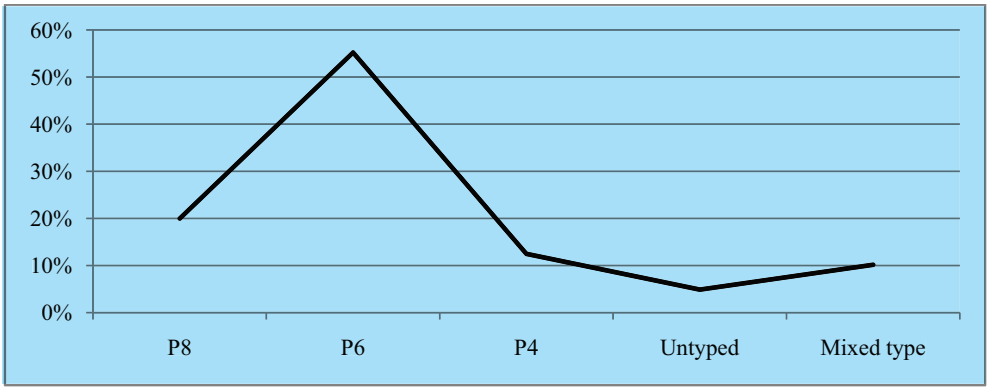

Figure 3: Distribution of P genotypes among rotavirus infected children

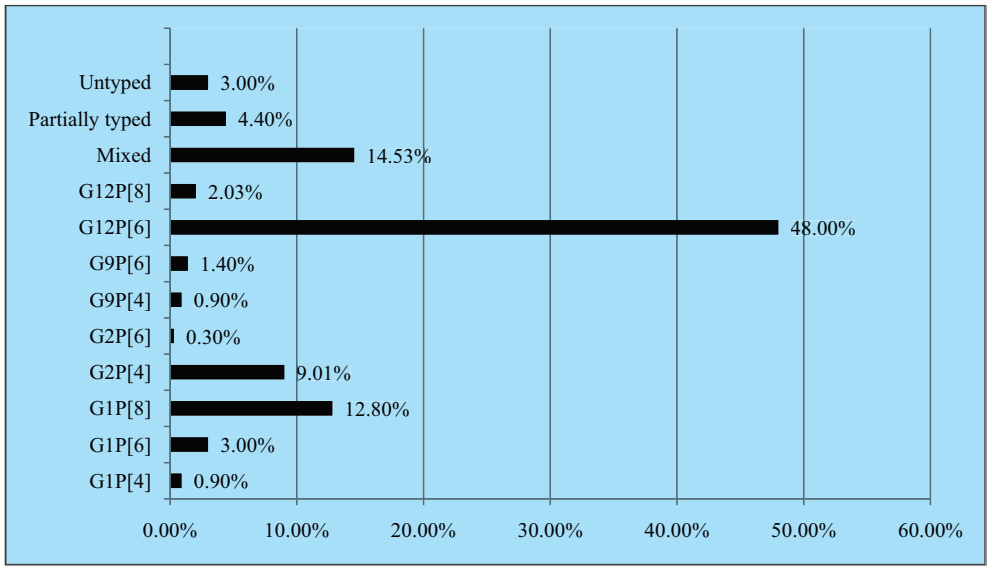

Figure 4: Distribution of G/P genotypes combination among rotavirus infected children (n=344)

Single G and P mixed genotypesinfections

Mixed infection with more than one G type was 22 cases in which G1-G12 (40.9%), G2-G12 (27.3%), G2-G9 (9.09%), G1-G2 (4.54%), G1-G2-G12 (13.6%) and G1-G2-G9-G12 (4.54%). Mixed P type infections were found 24 cases in which P[4]- P[6] (45.8%), P[6]-P[8] (20.8%), P[4]-P[8] (8.3%) and P[4]-P[6]-P[8] (25%)as shown in table 3. In addition, 18 untyped G genotypes and 13 were P genotypes were identified which challenges for further study.

Table 3: Distribution of Rotavirus single G and P mixed infection strain

Rotavirus mixed infection strain single G and single P and mixed genotyped			
Single G and mixed type strain (%)		Single P and mixed type strain (%)	
G1G12	9 (40.9)	P4 P6	11(45.8)
G2 G12	6 (27.3)	P6 P8	5 (20.8)
G1G2 G12	3 (13.6)	P4 P8	2 (8.3)
G2G9	2 (9.1)	P4 P6 P8	6 (25.0)
G1G2	1 (4.54)		
G1G2G9G12	1 (4.54)		
Total	**22**	**Total**	**24**
Untyped	18	Untyped	13

Discussion

Human rotaviruses are the major etiological agents of acute gastroenteritis in infants and young children worldwide[11].By following the previous trend[12-6] rotavirus was seen highest in winter season; in month January (58.8%) and among age group 12-23 months having nausea as commonest symptoms(40.8%) and vomiting (39.6%).

Globally, viruses carrying genotypes G1 to G4 and [P4] or[P8] have consistently been found to be the most commoncauses of rotavirus disease in humans, and different surveysindicate that [P8]G1, [P4]G2, [P8]G3, and [P8]G4 are the most common G and P types[17]. But in our study the most prevalent genotype was G12 and P6 which was in accordance with our previous study[12-6,18]. Since, the vast majority of G12 rotavirus strains has been isolated in Asia and in Southeast Asia[19] and it was assumed that from Southeast Asia, they might be transported across the globe by the increasing mobility of humans and animals. Following the G12, G1 is second commonest genotype that was found in this study, despite the fact that it was the leading causative agent worldwide[20-1]. G2 was found to be the third commonest genotype. G9 has been recognized as the most emerging genotype and was first reported in United States in the early 1980s and today it comprises4.1% of global rotavirus infections, and accounts for as high as 70% of rotavirus infections in some recently published data[22-3]. In the present study, G9 accounted for 8.1% of rotavirus diarrhea cases. This finding is similar to a previous report24. G9 was first discovered in Central America as a combination G9P8, which was considered a strain of epidemic emergency in the last few years in several countries in children with diarrhea[25-32]. The increase in reports of G9 from developed and developing countries shows the need for continued surveillance to identify the persistence of G9. Effectiveness of new available rotavirus vaccines against genotype G9 and the extent to which specific immunity plays role is still under discussion[33].In our study G3 genotype was not detected.

Four different G types were detected: G1, G2, G9 and G12. In this study G12 was the most prevalent genotype (52.3%), followed by G1 (17.7%), G2 (10.17%) and G9 (8.1%). Three different P types were detected: P4, P6 and P8. The P types identified were P[6] (55.23%), P[8] (20%), and P[4] (12.5%). These findings were similar to previous published data, but in contrast to other studies done in different countries the predominance of the P8or P4 genotype was seen[34].

A global rotavirus surveillance study indicated that the G/P combinations most frequently reported in humans were G1P[8], G3P[8], G4P[8], G2P[4], G9P[8], and G9P[6]23. But in our finding the trend was slightly deviated asG12P[6] (48%), G1P[8] (12.8%), G2P[4] (9%), G1P[6] (3%), G12P[8] (2.03%), G9P[6] (1.4%), G9P[4] and G1P[4] holding same (0.9%) and G2P[6] (0.3%). This was in accordance with other study done[12-4].

Since rotavirus vaccine was introduced, the genotype distribution has continued to fluctuate annually and geographically[11].Currently there are two rotavirus vaccines available. One of them is a monovalent rotavirus G1P8 vaccine while the other one is a pentavalentrotavirus vaccine covering G1, G2, G3, G4 and P8. This study showed a case of G12P6, G12P8, G9P4 and G9P6 infection in sample children. These serotypes are not a part of the vaccine serotypes. This study revealed the considerable serodiversity among human rotaviruses in this geographical region and hence emphasizes the need to give protection with multivalent rotavirus vaccines.

Mixed infections have been reported in about 14.5% of isolated rotavirus strains. In developing countries, the lack of adequate sanitation and proper water supplies and the

close association of humans with domestic animals could give rise to reassortment between human and animal strains with the potential of cross-species infections[35]. The prevalence of mixed infections (G type, 6.39%; P type, 10.17%) was similar to developing countries such as India, Bangladesh and Brazil[36-8] but little higher than the developed countries[39-41]. The probability and outcome of mixed infections with two different rotavirus strains may depend on both the frequency of each strain co-circulating in the population, and possible interference phenomena during dual replication in the gut[42]. Different types of reassortant rotaviruses were found to be grossly proportional with the numbers of observed relevant mixed infections. Whereas partially typed (4.4%) and untyped (3%) strains were also found in this study. The noticeable frequency of non-typeable and partially typed genotypes indicates the necessity of using another primer for characterization of unusual genotypes.

Conclusion

There is a high burden of rotavirus gastroenteritis among children less than five years of age. The variety distribution of G and P genotypes and electropherotypes of rotaviruses circulating in children were highlighted in the study. Emergence of G12 and G9 strains proves the immediate need of vaccine in Nepal.

Conflict of interests: None declared.

Acknowledgements

The authors would like to acknowledge SEARO-WHO for supporting this study. We are grateful to Professor Gagandeep Kang, The Wellcome Trust Research Laboratory CMC Vellore WHO collaborating center for confirmation of genotyping samples.

References

1. Donatoa C. Molecular epidemiology of rotavirus in the era of vaccination. Microbiol. Australia. 2012; Pp-64-6
2. Husain M, Seth P, Dar L, and Broor S. Classification of Rotavirus into G and P Types with Specimens from Children with Acute Diarrhea in New Delhi, India. J of ClinMicrobiol. 1996; 34(6):1592–4
3. Rahman M, Sultana R, Ahmed G, Nahar S, Hassan ZM, Saiada F, Podder G, Faruque ASG, Siddique AK, Sack DA, Matthijnssens J, Ranst MV, and Azim T. Prevalence of G2P[4] and G12P[6] Rotavirus, Bangladesh. Emerg Infect Dis. 2007; 13(1);18-24
4. Chakravarti A, Chauhan MS, Sharma A, Verma V. Distribution of human rotavirus G and P genotypes in a hospital setting from Northern India.Southeast Asian J Trop Med Public Health. 2010; 41(5):1145-52.
5. Bonkoungou IJO, Damanka S, Sanou I, Tiendre´be´ogo S, Coulibaly SO, Bon F, Haukka K, Traore AS, Barro N, and Armah GE. Genotype Diversity of Group A Rotavirus Strains in Children With Acute Diarrhea in Urban Burkina Faso, 2008–2010. J of Med. Virol.2011; 83:1485–90
6. Mascarenhas JDP, Gusmão RHP, Barardi CRM, Paiva FL, Simões CO, Gabbay YB, Monteiro TAF &Linhares AC. Characterization of rotavirus P genotypes circulating among paediatric inpatients in northern Brazil. Inst. Med. trop.1999; 41(3): 165-70.
7. Gentsch JR, Woods PA, Ramachandran M. et al. - Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J. Infect. Dis. 1996; 174(1):30-6.
8. Timenetsky MC, Santos N &Gouvea V. Survey of rotavirus G and P types associated with human gastroenteritis in São Paulo, Brazil, from 1986 to 1992. J. lin. Microbiol. 1994; 32: 2622-4.
9. Nakawesi JS, Wobudeya E, Ndeezi G, Mworozi EA and Tumwine JK. Prevalence and factors associated with rotavirus infection among children admitted with acute diarrhea in Uganda. Nakawesi et al. BioMed Central Pediatrics. 2010; 10:(62):2-5
10. Bourdett-Stanziola L, Jiménez CDR, and Ortega-Barria E. Diversity of Human Rotavirus G and P Genotypes in Panama, Costa Rica, and the Dominican Republic American J Trop. Med. Hyg. 2008; 79(6):921–4
11. Mohammad K, Maryam Z, and Akram N. Molecular Epidemiology of Rotavirus Strains Circulating among Children with Gastroenteritis in Iran. Iran J Pediatr. 2012; 22(1):63-9
12. Sherchand JB, Nakagomi O, Dove W, Nakagomi T, Yokoo M, Pandey BD, Cuevas LE, Hart A and Cunliffe NA. Molecular epidemiology of rotavirus diarrhea among children aged <5 years in Nepal: Predominance of emergent G12 strains during 2 years. J Infect Dis. 2009; 200:4222-6.
13. Pun SB, Nakagomi T, Sherchan JB, Pandey BD, Cuevas LE, Cunliffe NA et al. Detection of G 12 human rotavirus in Nepal. Emerg Infect Dis. 2007; 13:482-83.
14. Uchida R., Pandey BD, Sherchand JB, Ahmed K, Yokoo M, Nakagomi T et al. Molecular epidemiology

of rotavirus diarrhea among children and adults in Nepal: detection of G12 strains with P[6] or P[8] and a G11P[25] strain. J ClinMicrobiol. 2006; 44:3499–505.

15. Sherchand JB, Cunliffe NA, Tandukar S, Yokoo M, Pandey BD, Niraula P, Panta AR, Nakagomi O. Rotavirus Disease Burden and Molecular Epidemiology in Children with Acute Diarrhea Age Less than 5 Years in Nepal. J Nepal PaediatrSoc 2011; 31:215-18

16. Parashar UD, Holman RC, Clarke MJ, Bresee JS, and Glass RI. Hospitalizations Associated with Rotavirus Diarrhea in the United States, 1993 through 1995: Surveillance based New ICD-9-CM Rotavirus-Specific. J Infect Dis 1998; 177:13–7

17. Heide R, Koopmans MPG, Shekary N, Houwers DJ, Duynhoven YTHP, and Poel WHMN. Molecular Characterizations of Human and Animal Group Rotaviruses in the Netherlands. Journal of clinical microbiology. 2005; 43(2):669–5

18. Ansari S, Sherchand JB, Rijal BP, Parajuli K, Mishra SK, Dahal RK, Shrestha S, Tandukar S, Chaudhary R, Kattel HP, Basnet A and Pokhrel BM. Characterization of rotavirus causing acute diarrhoea in children in Kathmandu, Nepal, showing the dominance of serotype G12. J of Med Microbioly. 2013; 62:114–120.

19. Rahman M, Matthijnssens J, Yang X, Delbeke T, Arijs I, Taniguchi K, Iturriza-Gómara M, Iftekharuddin N, Azim T, and Ranst MV. Evolutionary History and Global Spread of the Emerging G12 Human Rotaviruses. J Virol. 2007; 81:2382–90.

20. Argüelles MH, Villegas GA, Castello A, Abrami A, Ghiringhelli PD, Semorile L, Glikmann G. VP7 and VP4 genotyping of human group A rotavirus in Buenos Aires, Argentina. J Clin.Microbiol. 2000; 38:252–259

21. Bon F, Fromantin C, Aho S, Pothier P, Kohli E, and The Azay group. G and P genotyping of rotavirus strains circulating in France over a three-year period: detection of G9 and P [6] strains at low frequencies. J ClinMicrobiol 2000; 38:1681–3

22. Kirkwood CD et al. Report of the Australian Rotavirus Surveillance Program, 2001/2002. Commun. Dis Intell 2000; 26:537–40.

23. Santos N and Hoshino Y. Global distribution of rotavirus serotypes/ genotypes and its implications for the development and implementation of an effective rotavirus vaccine. Rev Med Virol 2005; 15:29–56.

24. Banerjee I, Ramani S, Primrose B, Moses P, Iturriza-Gomara M, Gray J, Jaffar S, Monica B, Muliyil J, Brown D, Estes M, Kang G. Comparative study of the epidemiology of rotavirus in children from a community-based birth cohort and a hospital in South India. J ClinMicrobiol 2006; 44:2468–74.

25. Cunliffe NA, Kilgore PE, Bresee JS, Steele AD, Luo N, Hart CA, Glass RI. Epidemiology of rotavirus diarrhoea in Africa: a review to assess the need for rotavirus immunization. Bull WHO1998; 76:525–37.

26. Cunliffe NA, Bresee JS, Hart CA. Rotavirus vaccines: development, current issues and future prospects. J Infect 2002; 45:1–9.

27. Estes MK. Rotaviruses and their replication. In: Fields BN, Knipe DM, Howley PM et al. (eds) Fields' Virology 4th ed. Lippincott Williams and Wilkins, Philadelphia. 2001; 1747–58

28. Fang ZY, Yang H, Jin Q, Jing Z, Sun LW S,Tang JY, Ma L, Du ZQ, He AH, Xie JP, Lu YY, Ji ZZ, Zu BQ, Wu HY, Lin SE, Xie HP, Griffin DD, Ivanoff B, Glass RI, Gentsch JR. Diversity of rotavirus strains among children with acute diarrhea in China: 1998–2000 surveillance study. J ClinMicrobiol 2002; 40:1875–78

29. Fruhwirt M, Brosl S, Ellemunter H, Moll-Schüler I, Rohwedder A, Mutz I. Distribution of rotavirus VP4 genotypes and VP7 serotypes among nonhospitalized and hospitalized patients with gastroenteritis and patients with nosocomially acquired gastroenteritis in Austria. J ClinMicrobiol 2000; 38:1804–06

30. Gentsch JR, Woods PA, Ramachandran M, Das BK, Leite JP, Alfieri A, Kumar R, Bhan MK, Glass RI. Review of G and P typing results from a global collection of strains: implications for vaccine development. J Infect Dis 1996; 174:30–36

31. Jain V, Das BK, Bhan MK, Glass RI, Gentsch JR and The Indian Strain Surveillance Collaborating Laboratories. Great diversity of group A rotavirus strains and high prevalence of mixed rotavirus infection in India. J ClinMicrobiol 2001; 39:3524–9

32. Masendycz PJ, Unicomb LE, Kirkwood CD, Bishop RF. Rotavirus serotypes causing severe acute diarrhea in young children in six australian cities, 1989 to 1992. J ClinMicrobiol 1994; 32:2315–7

33. Bourdett-Stanziola L, Jiménez C, and Ortega-Barria E. Diversity of Human Rotavirus G and P Genotypes in Panama, Costa Rica, and the Dominican Republic. Am J Trop Med Hyg 2005; 79:921–4

34. Bahl R, Ray P, Subodh S, Shambharkar P, Saxena M, Parashar U, Gentsch J, Glass R, and Bhan MK . Incidence of severe rotavirus diarrhea in New Delhi, India, and G and P types of the infecting rotavirus strains.J Infect Dis 2005; 192 (1):114-9

35. Cook N, Bridger J, Kendall K, Iturriza-Go´mara M, El-Attar L, and Gray J. The zoonotic potential of rotavirus. J Infect 2004; 48:289–302.

36. TimenetskyMC,Santos N, Gouvea V. Survey of rotavirus G and P types associated with human gastroenteritis in Sao Paulo, Brazil, from 1986 to 1992. J ClinMicrobiol 1994; 32:2622–4.

37. Unicomb LE, Podder G, Gentsch JR, Woods PA, Hasan KZ, Faruque AS, Albert MJ, Glass RI. Evidence of high-frequency genomic reassortment of group A rotavirus strains in Bangladesh: Emergence of type G9 in 1995. J ClinMicrobiol 1999; 37:1885–91.

38. Jain V, Das BK, Bhan MK, Glass RI, Gentsch JR, Indian Strain Surveillance Collaborating Laboratories. Great diversity of group A rotavirus strains and high prevalence of mixed rotavirus infections in India. J ClinMicrobiol 2001; 39:3524–9.

39. Gentsch JR, Woods PA, Ramachandran M. et al. - Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J Infect Dis1996; 174:30-36.

40. Griffin DD, Kirkwood CD, Parashar UD, Woods PA, Bresee JS, Glass RI, Gentsch JR. Surveillance of rotavirus strains in the United States: Identification of unusual strains. The National Rotavirus Strain Surveillance System collaborating laboratories. J ClinMicrobiol 2000;38:2784–7.

41. Iturriza-Gomara M, Cubitt D, Steele D, Green J, Brown D, Kang G, Desselberger U, Gray J. Characterisation of rotavirus G9 strains isolated in the UK between 1995 and 1998. J MedVirol 2000; 61:510–7.

42. Ward RL and Bernstein DI. Rotarix: a rotavirus vaccine for the world. Clin Infect Dis 2009; 48:222–8.