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Molecular Characterization of Circulating Rota Viral Strain causing infantile diarrhoea in Tripura, North East India

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Abstract

Introduction: *North Eastern India is geographically distinct from rest of India and the trend of Rota viral diarrhoea among children below 5 years of age group is not well studied yet. The Government of India introduced the oral rotavirus vaccine (ROTAVAC) in 2016 as part of the Universal Immunization Programme in different parts of India.* ***Objective:*** *(1). To determine the proportion of circulating Rotavirus strains causing acute viral gastroenteritis (AGE) among children below 5 years of age group. (2). Molecular characterization of Rotavirus genotypes. (3). Impact of vaccination on pattern of circulation of different Rotavirus genotype.* ***Methods****: Stool samples were collected between August 2016 to May 2019 and subjected to VP6 antigen detection of Group A Rotavirus by ELISA followed by genotyping by Semi-nested Polymerase Chain Reaction. The statistical analysis was done using Graph pad Prism 9. The categorical variables were distributed as proportions and the p-value was calculated by using Pearson's chi-square test and Fisher's exact test. p-Value of <0.05 was considered statistically significant.* ***Results****: Among the diarrheal episodes of the study subjects 39.3% was positive for rotavirus. G3P(8) was the predominant genotype followed by G1P(8). These genotypes alone contributed 51.1% and 23.8% respectively among the positive cases. A relatively new genotype, G9P(4) emerged during 2018–2019 with a prevalence of 6.8% that was not noticed during 2016–2017.* ***Conclusion****: This study highlights the importance of monitoring the trend of circulating rotavirus strains, which may help in appropriate management and control of Rotaviral diarrhoea among paediatric population*

Keyword: Acute gastroenteritis, Rotavirus, Circulating Genotype, Semi-nested PCR, ROTAVAC

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INTRODUCTION

Rotavirus is a double-layered, non-enveloped RNA virus, a member of *Reoviridae* family [1, 2]. They are classified into seven groups from A to G. However globally Group A Rotavirus is commonly associated with severe acute gastroenteritis among infants and children in developing and industrialized countries [3].

The virus has a characteristic wheel-like appearance consisting of crucial structural (VP1-VP4, VP6, and VP7) proteins. Its genome consists

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of 11 segments of double stranded RNA [4, 5]. The structural protein; VP6 determines the antigenic specificity and subgroup classification of rotavirus. The two outer layer proteins, VP7 and VP4 determine the serotype specificity and are involved in dual system classification of the virus. VP4 is a non-glycosylated protease-sensitive protein and VP7 is a glycoprotein. Both VP4 and VP7 respectively, determine the P and G serotypes of the virus. Due to the independent segregation of VP4 and VP7, different G and P combinations are frequently observed in natural infections. The prevalence of G1P(8), G2P(4), G3P(8), G4P(8), G9P(6), and G9P(8) in human, along with other combinations have been well documented in several reports [6, 7]. Rotavirus alone contributes 39% of diarrheal cases globally among hospitalized children. In India, 11.37 million episodes of AGE in children below 5 years are caused by Rotavirus annually [8]. During 2005–2009, the Indian Rotavirus Strain Surveillance Network assessed that 40% of AGE is related with Rotavirus among children below 5 years [8]. Other studies, Babji. S. et al., between 2009–2012 have supported 26–39% rotavirus associated AGE cases among children [9, 10]. In April 2016, under the Universal Immunization Programme (UIP), the Government of India introduced the ROTAVAC vaccine containing the live 116E rotavirus strain G9P(11), among different states of India for the prevention of rotavirus associated AGE [11]. Tripura introduced ROTAVAC in the month of February 2017 by the Honourable Central Minister of Health and Family Welfare. In Tripura, 'Study of molecular characterization of Rotavirus' was undertaken in the year 2016 for a period of three (3) years. The objective of the study was to estimate the proportion of diarrhoea attributed to Rota virus. The study was focused on the children <5 years of age in hospitals and community setups along with their genotypic characterization and also seasonal distribution. The study output would help in understanding the dynamics of the disease trend and also the impact of vaccination in reducing the disease burden in the State of Tripura.

METHODS

Study Population

The study population was children ≤5 years of age, hospitalized with more than 3 episodes of watery diarrhoea in 24 hrs. Informed consent form from the parents was obtained before each of the enrolled children. Cases with features of dysentery, less than 3 episodes of diarrhoea in 24 hrs and more than 5 years of age group were excluded.

Ethical Approval and Consent to Participate

The methodology for collecting patient sample and information were approved by the Institutional Ethical Committee for Clinical Studies of Agartala Government Medical College, vide number: Ref. No. F. 4(5-192)/AGMC/Academic/IEC Meeting/2015/9946, dated: 15.07.2015.

Sample Collection and Transport

All the stool samples were collected in properly labelled sterile containers. It was transported to the laboratory within two hours of collection or stored at 4°C till transported. After reaching the laboratory, 10% (ten present) (W/V) stool suspension was prepared using 1X Dulbecco's PBS and centrifuged. The supernatant thus obtained was used for VP6 antigen detection and genotyping of the samples.

Laboratory Procedures

Antigen Detection

All the stool samples were preliminarily screened for the presence of VP6 antigen [1, 12] of Rotavirus with the help of Premier™ Rotaclone ELISA Kit by Meridian Biosciences according to the manufacturer's protocol. Spectrophotometry based Multiskan ELISA reader was used to determine the results at 450 nm. Samples with O.D. values >0.15 were considered as positive.

Viral RNA extraction

For viral RNA extraction, 30%(v/v) suspensions of Rotavirus antigen positive samples were prepared by the addition of 300 µl of stool sample in 700 µl of sterile Dulbecco's Phosphate Buffered

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Saline (10X). The sample suspensions were vortexed thoroughly for 1 min and centrifuged at 10,000 rpm for 10 minutes at 4°C. The clear supernatants were collected and used for RNA extraction by QIAamp 96 Virus QIAcube HT Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions [13].

G and P typing

For the determination of G (VP7) and P (VP4) genotypes, the extracted RNAs were subjected to Reverse Transcription (RT)-PCR for the synthesis of complementary DNA (cDNA) using random primers (Invitrogen). Synthesized cDNAs were further amplified using the standard primer sets (Table 1) in Semi-nested PCR. The reaction volume was 50 µl in the two rounds of PCR. In the 1st round, 5 µl of the viral cDNA was mixed with 45 µl of the PCR master mix containing 1 µl of the forward and reverse primers, 10X buffer (Invitrogen), 50 mM $MgCl_2$ (Qiagen), 10 mM dNTPs(Qiagen), 5 U/µlTaq polymerase (Invitrogen) and RNase-free H_2O (Qiagen) [13].

In the 2nd round, 2 µl of the 1st round PCR product was mixed with 48 µl of PCR master mix containing 10X buffer (Invitrogen), 50 mM $MgCl_2$ (Qiagen), 10 mM dNTPs (Qiagen), 5 U/µlTaq polymerase (Invitrogen) and RNase-free H_2O (Qiagen) and 1 µl of the specific primers. The cycling condition consisted of initial denaturation at 94°C for 4 mins followed by 30 cycles of PCR and a final extension at 72°C for 7 mins [14].

Gel Electrophoresis

The PCR amplicons were electrophoresed on a 2% agarose gel prepared in a TAE buffer stained with ethidium bromide. The PCR products were loaded in the gel wells along with 1 kB plus DNA Ladder for Rotavirus and 100 bp plus DNA Ladder for Non-rota virus panel. The amplicon size was compared with the Ladder size by visualizing under the UV-Transilluminator [13].

Statistical Analysis

All the forms that were collected checked properly and the data were maintained in the Excel sheet. The statistical analysis was done using Graph pad Prism 9. The categorical variables were distributed as proportions and the p-value was calculated by using Pearson's chi-square test and Fisher's exact test. p-value of <0.05 was considered statistically significant.

RESULTS

During the study period a total number of 645 samples were collected as per the inclusion criteria. Out of 645 samples, 39.3% (254/645) were positive for the presence of Rota viral antigen (RVA) (Figure 1).

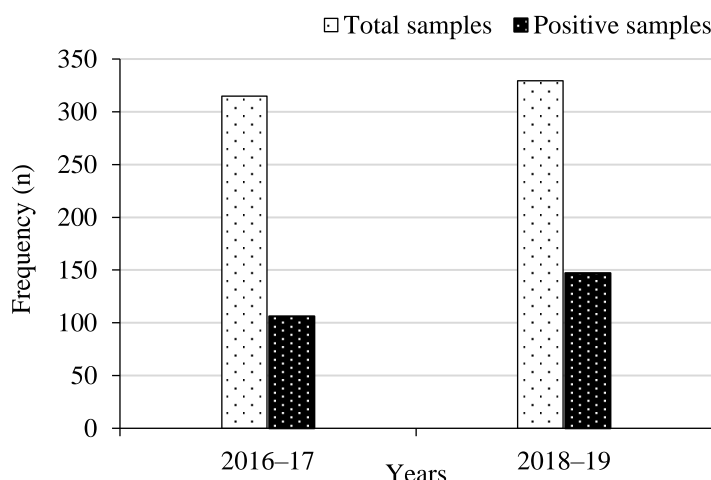

Figure 1. Positivity for presence of Rota viral antigen (RVA).

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The sex distribution of the positive samples shows a ratio of 2:1 in male to female. RVA detection among males was 61.4% (n= 156) compared to females 38.5% (n= 98). Age distribution of the study subjects shows positivity among 0 to 1 year of age group was 54.7% (n= 139) followed by 1.1-2 years, which is 20.0% (n= 51) (Figure 2 and Table 1).

The month wise positivity of Rota-virus infection shows a peak during winter season, though antigen could be detected throughout the year. During 3 (three) years of study period, it was found that infection rate was higher in second year, as compared to first and third year (Figure 3).

Genotyping characterization of ELISA positive samples were done by semi-nested PCR as described by WHO [4]. Both G and P serotypes were targeted and different combinations of G and P serotypes were observed. The common circulating VP7 serotypes observed throughout the study period were G3 and G1, whereas P(8) contributed the most among the VP4 serotypes. G3P(8) contributed the most among the circulating genotypes (51.1%(130/254)) followed by G1P(8) (24%(61/254)). During the study period, a number of other combinations viz. G12P(8) (4.3%), G9P(4) (3.9%), G2P(4) (3.1%) were also observed in lower proportion. A small fraction of the positive samples showed mixed typing (4.3%) such as G1G12P(8) (1.5%), G3G12P(8) (2.7%), while a few were partially typed (7.8%) for both G and P serotypes (Figure 4 and Table 2).

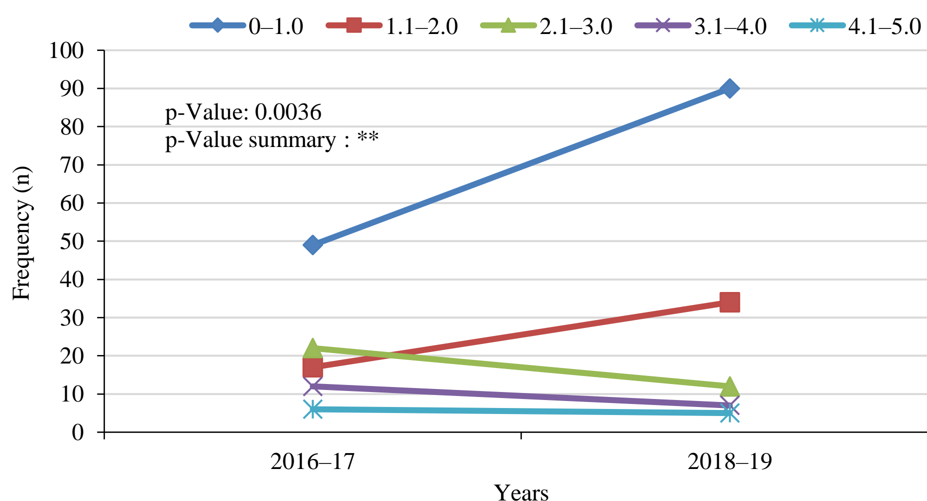

Figure 2. Age wise distribution of RVA.

Table 1. Socio-demographic survey of the study population.

Variables	2016–2017 N (%)	2018–2019 N (%)	Total N (%)	*p*-value
Sex				
Male	74 (69.8)	82 (55.4)	156 (61.4)	0.013
Female	32 (30.1)	66 (44.5)	98 (38.5)	
Age group (years)				
0–1.0	49 (46.2)	90 (60.8)	139 (54.7)	0.0036
1.1–2.0	17 (16.0)	34 (22.9)	51 (20.0)	
2.1–3.0	22 (20.7)	12 (8.1)	34 (13.3)	
3.1–4.0	12 (11.3)	7 (4.7)	19 (7.4)	
4.1–5.0	6 (5.6)	5 (3.3)	11 (4.3)	
VP6 antigen detection results				
ELISA Positive	106 (33.6)	148 (44.8)	254 (39.3)	
ELISA Negative	209 (66.3)	182 (55.1)	391 (60.6)	

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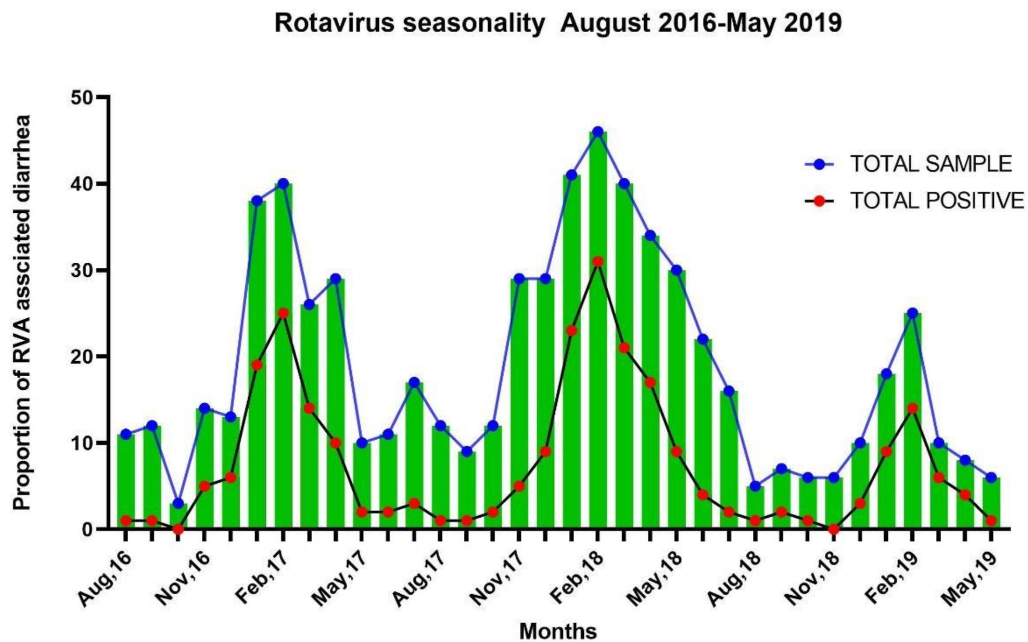

Figure 3. Month wise positivity distribution of RVA-Associated diarrhoea from August 2016 to May 2019. Graph Shows the peak of RVA detection During February each year.

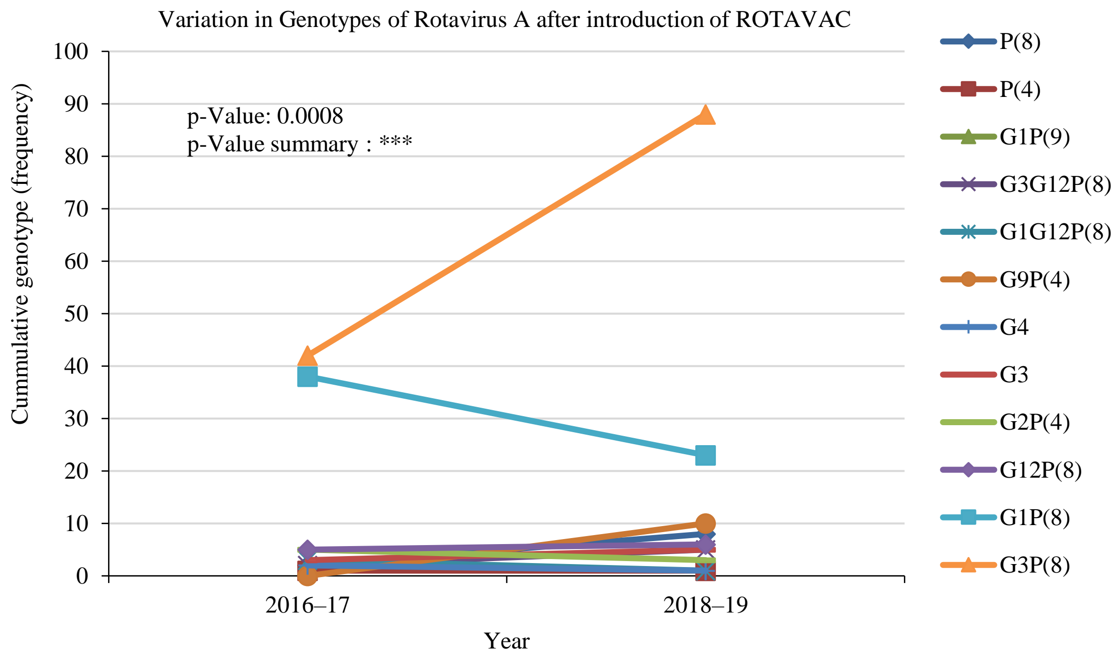

Figure 4. Diversity in rotavirus genotypes in the early phase of post vaccination.

Following introduction of vaccination in February 2017 a shift in circulation genotypes were observed among the positive samples. Though during 2016–2017 the predominating genotypes were G3P(8) (39.6%) and G1P(8) (35.8%). However during 2018–2019, the proportion of G3P(8) increased up to 59.5%, while G1P(8) showed a sharp decline (15.5%). Moreover, G1P(9) constituted 2.8% during 2016–2017, which could not be detected post vaccination i.e. 2018–2019. A relatively new genotype, G9P(4) emerged during 2018–2019 with a prevalence of 6.8% that was not noticed during 2016–2017 (Figure 5).

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Table 2. Percentage distribution of RVA genotypes during the study period.

Genotypes	2016–17 N (%)	2018–19 N (%)	Total N (%)
G3P(8)	42 (39.6)	88 (59.5)	130 (51.1)
G1P(8)	38 (35.8)	23 (15.5)	61 (24.0)
G12P(8)	5 (4.7)	6 (4.1)	11 (4.3)
G2P(4)	5 (4.7)	3 (2.0)	8 (3.1)
G9P(4)	0 (0.0)	10 (6.8)	10 (3.9)
G1P(9)	3 (2.8)		3 (1.1)
Mixed typed			11 (4.3)
G1G12P(8)	3 (2.8)	1 (0.7)	4 (1.5)
G3G12P(8)	2 (1.9)	5 (3.4)	7 (2.7)
Partially typed			20 (7.8)
VP7:			
G3	3 (2.8)	5 (3.3)	8 (3.1)
G4	2 (1.9)	1 (0.7)	3 (1.1)
VP4:			
P(4)	1 (0.9)	1 (0.7)	2 (0.7)
P(8)	2 (1.9)	5 (3.4)	7 (2.7)

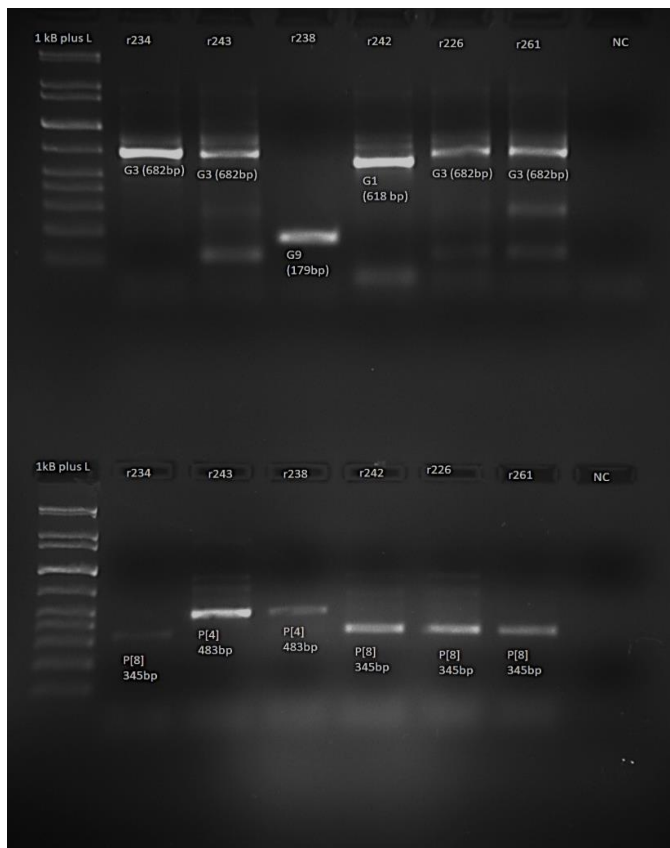

Figure 5. Agaraose gel electrophoresis showing different amplified products of VP7 (G) and VP4(P) genes. Lane 1 contains 1 kb Plus Ladder, Lane 2–7 contains different ELISA positive samples, Lane 8 contains PCR master mix as negative control.

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DISCUSSION

This study shows the Rota viral diarrhoea plays a significant role among the children below 5 years of age. The study found 39.33% of Rota viral diarrhoea among all diarrhoeal cases is similar to earlier reviewed studies that showed 34% of all AGE among children in India to be associated with Rotavirus alone [14]. The preponderance of Rota viral diarrhoea among 0 to 1 year age group children is also similar to the other studies reported in India [15]. Though the RVA could be detected throughout the year, a seasonal trend has been observed during the month December to March i.e. during winter and also early summer which is similar to the other studies [16]. Molecular characterization of Rota virus shows simultaneous circulation of multiple genotypes, the highest being G3P(8), followed by G1P(8), G12P(8), G2P(4), G9P(4), G1P(9). The mixed type variants were observed among 4.3% of the cases, comprising of G1G12P(8), G3G12P(8) which indicates the probability of acquiring the infection from different sources and might serve as emergence of a novel strain as this virus has a segmented RNA genome. Among the partially typed strains that comprises 7.8% of the study cases, G3 and G4 were common among the VP-7 serotypes and P(4), P(8) and among the VP4 serotypes. Sequencing of the respective isolates would help in understanding the exact pattern of circulating strains of rotavirus. The rolling out of ROTAVAC under the universal immunization program was launched in Tripura by the Honourable Central Minister of Health and Family Welfare in the month of February 2017. Additional analysis of the genotypic pattern characterized pre and post vaccination shows there is a significant shift in the circulating strains. Though during 2016–2017, the predominating genotypes were G3P(8) (39.6%) and G1P(8) (35.8%) however, during 2018–2019, the proportion of G3P(8) increases up to 59.5%, while G1P(8) showed a sharp decline (15.5%). Moreover, G1P(9) constituted 2.8% during 2016–2017, which could not be detected post vaccination i.e. 2018–2019. A relatively new genotype, G9P(4) emerged during 2018–2019 with a prevalence of 6.8% that was not noticed during 2016–2017. During the study period of 3 years, G3P(8) and G1P(8) remained predominant among the circulating strains. Other studies in India have also reported these genotypes among the common circulating strains of rotavirus [1]. However, unlike G1P(8) which tends to be the most common circulating strain in India, G3P(8) appeared more frequently among the rotavirus associated AGE in Tripura. G3P(8) constitute 51.1% of the infected cases whereas G1P(8) constitute 24.0%. The circulation of other variants of rotavirus genotypes such as G2P(4), G12P(8), G1P(9) were also prominent in Tripura. An emergence of a new strain G9P(4) was observed after the introduction of ROTAVAC. Globally, G9 is the fifth most common VP7 serotype and was first reported in Philadelphia in 1983 [14]. The emergency of G9P(4) and disappearance of G1P(9) certainly signifies a rapid change in the circulating rotavirus strain on introduction of ROTAVAC. The result of the study signifies circulation of multiple genotypes of Rotavirus in the State of Tripura. It is playing a significant role in causing acute diarrhoeal episodes among the children < 5 years. This study helps us to understand the need for on-going surveillance on Rota virus and its circulating genotypes along with the vaccination programme. It also further emphasises the need of sequencing for mixed type and the un-typable strains. This study also helps the paediatricians in proper management of acute viral diarrhoea, which indirectly will limit the injudicious use of antibiotics among the patients with acute diarrhoea.

CONCLUSION

This study highlights the high prevalence of Rota viral infection among diarrheal diseases in the paediatric population. Multiple genotypes are in circulation in the state of Tripura. The G3P(8) and G1P(8) remained the most common genotypes and the study also highlights the emergence of G9P(4) post vaccination period. The study further emphasises the need of sequencing for mixed type and the un-typable strains. The on-going surveillance should be continued for monitoring and effective management of Rota viral diarrheal diseases.

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REFERENCES

1. Giri, S., Nair, N.P., Mathew, A. et al. Rotavirus gastroenteritis in Indian children < 5 years hospitalized for diarrhoea, 2012 to 2016. BMC Public Health 19, 69 (2019). https://doi.org/10.1186/s12889-019-6406-0.
2. Jacob John, Rajiv Sarkar, Jayaprakash Muliyil, et al., Rotavirus gastroenteritis in India, 2011–2013: Revised estimates of disease burden and potential impact of vaccines, Vaccine, Volume 32, Supplement 1,2014,Pages A5-A9,ISSN 0264-410X,https://doi.org/10.1016/j.vaccine.2014.03.004.
3. Bhandari N, Rongsen-Chandola T, Bavdekar A, et al. Efficacy of a monovalent human-bovine (116E) rotavirus vaccine in Indian infants: a randomised, double-blind, placebo-controlled trial. Lancet. 2014;383(9935):2136-2143. doi:10.1016/S0140-6736(13)62630-6.
4. World Health Organization. Manual of rotavirus detection and characterization methods. Geneva: World Health Organization; 2009.https://apps.who.int/iris/bitstream/handle/10665/70122/WHO_IVB_08.17_eng.pdf?sequence=1&isAllowed=y. Accessed on 15th March 2021.
5. Gouvea, V, R I Glass, P Woods et al. "Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens." Journal of clinical microbiology vol. 28,2 (1990): 276-82. doi:10.1128/JCM.28.2.276-282.1990.
6. Grimwood, K., Lambert, S.B. & Milne, R.J. Rotavirus Infections and Vaccines. Pediatr-Drugs 12, 235–256 (2010). https://doi.org/10.2165/11537200-000000000-00000.
7. Van Doorn LJ, Kleter B, Hoefnagel E, et al. Detection and genotyping of human rotavirus VP4 and VP7 genes by reverse transcriptase PCR and reverse hybridization. J ClinMicrobiol. 2009;47(9):2704-2712. doi:10.1128/JCM.00378-09.
8. Saluja T, Dhingra MS, Sharma SD, et al. Association of rotavirus strains and severity of gastroenteritis in Indian children. Hum Vaccin Immunother. 2017;13(3):711-716. doi:10.1080/21645515.2016.1238994.
9. Kang G, Desai R, Arora R, et al. Diversity of circulating rotavirus strains in children hospitalized with diarrhea in India, 2005-2009. Vaccine. 2013;31(27):2879-2883. doi:10.1016/j.vaccine.2013.04.030.
10. Babji S, Arumugam R, Sarvanabhavan A, et al. Multi-center surveillance of rotavirus diarrhea in hospitalized children <5 years of age in India, 2009-2012. Vaccine. 2014;32Suppl 1:A10-A12. doi:10.1016/j.vaccine.2014.03.001.
11. Saluja T, Sharma SD, Gupta M, et al. A multicenter prospective hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children less than five years of age in India. Vaccine. 2014;32Suppl 1:A13-A19. doi:10.1016/j.vaccine.2014.03.030.
12. Boni-cisse, C., Meite, S., Mlan, A.B. et al. Genotypic characterization of rotavirus in children under 5 years circulating in Côte D'Ivoire from 2010 to 2013. Virol J 15, 78 (2018). https://doi.org/10.1186/s12985-018-0973-z.
13. Tate JE, Burton AH, Boschi-Pinto C, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis. 2012;12(2):136-141.
14. Kahn, G., Fitzwater, S., Tate, J. et al. Epidemiology and prospects for prevention of rotavirus disease in India. Indian Pediatr 49, 467–474 (2012). https://doi.org/10.1007/s13312-012-0076-7.
15. Anca IA, Furtunescu FL, Pleşca D, Streinu-Cercel A, Rugină S, Holl K. Hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children below five years of age in Romania. Germs. 2014;4(2):30-40. Published 2014 Jun 2. doi:10.11599/germs.2014.1053.
16. Rahman, M., Matthijnssens J, Yang X et al. Evolutionary history and global spread of the emerging g12 human rotaviruses. Journal of virology, 81(5), 2382–2390. https://doi.org/10.1128/JVI.01622-06.

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