

# Molecular Epidemiology of Rotavirus among Children in Western Canada: Dynamic Changes in Genotype Prevalence in Four Consecutive Seasons

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## Abstract

Rotavirus molecular surveillance remains important in the post vaccine era in order to monitor the changes in transmission patterns, identify vaccine induced antigenic changes and discover potentially pathogenic vaccine related strains. The Canadian province of Alberta introduced rotavirus vaccination into its provincial vaccination schedule June 2015. To evaluate the impact of this program on stool rotavirus positivity rate, strain diversity and seasonal trends, we analyzed a prospective cohort of children with acute gastroenteritis recruited between December 2014 and August 2018. We identified dynamic changes in rotavirus positivity and genotype trends during pre- and post- rotavirus vaccine introduction periods. Genotypes G9P[8], G1P[8], G2P[4] and G12P[8] predominated consecutively each season with overall lower rotavirus incidence rates in 2016 and 2017. The demographic and clinical features of rotavirus gastroenteritis were comparable among wild type rotaviruses; however, children with G12P[8] infections were older ( $P < 0.001$ ). Continued efforts to monitor changes in the molecular epidemiology of rotavirus using whole genome sequence characterization is needed to further understand the impact of the selection pressure of vaccination on rotavirus evolution.

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#### **Abstract :**

Rotavirus molecular surveillance remains important in the post vaccine era in order to monitor the changes in transmission patterns, identify vaccine induced antigenic changes and discover potentially pathogenic vaccine related strains. The Canadian province of Alberta introduced rotavirus vaccination into its provincial vaccination schedule June 2015. To evaluate the impact of this program on stool rotavirus positivity rate, strain diversity and seasonal trends, we analyzed a prospective cohort of children with acute gastroenteritis recruited between December 2014 and August 2018. We identified dynamic changes in rotavirus positivity and genotype trends during pre- and post- rotavirus vaccine introduction periods. Genotypes G9P[8], G1P[8], G2P[4] and G12P[8] predominated consecutively each season with overall lower rotavirus incidence rates in 2016 and 2017. The demographic and clinical features of rotavirus gastroenteritis were comparable among wild type rotaviruses; however, children with G12P[8] infections were older ( $P < 0.001$ ). Continued efforts to monitor changes in the molecular epidemiology of rotavirus using whole genome sequence characterization is needed to further understand the impact of the selection pressure of vaccination on rotavirus evolution.

#### **Significance:**

We identified dynamic changes in rotavirus molecular epidemiology in a prospective cohort of children with acute gastroenteritis during pre- and post- monovalent rotavirus vaccine (RV1) introduction era. While the circulating rotavirus genotypes remained mostly unchanged, strain predominance varied yearly. We found the emergent G12P[8] strain predominantly infected older children and was the most common strain in fully vaccinated children. Knowledge of the association between rotavirus genotype diversity and AGE clinical severity is limited. Our data suggest that other than RV1 related G1P[8] isolates, the clinical severity of different rotavirus genotypes did not differ significantly, thus emphasizing the need for rotavirus vaccines that protects against all genotypes.

#### **Introduction:**

Before the introduction of rotavirus vaccines, rotavirus gastroenteritis was a leading cause of childhood morbidity and mortality in children under five years of age worldwide (1, 2). To date, more than 100 countries have introduced rotavirus vaccines into their national immunization programs, significantly reducing the burden of rotavirus associated hospitalizations and deaths (3, 4). However, vaccine efficacy is higher in resource-rich countries and the societal impact of rotavirus gastroenteritis remains large. Rotavirus infections result in an estimated 120,000 to 200,000 deaths annually in resource-limited countries and causes significant societal and healthcare costs in resource-rich countries (5-7).

*Rotavirus*, a genus of the family *Reoviridae*, is a genetically diverse group of dsRNA viruses. Most cases of disease belong to group A rotavirus which can be classified into 42 G and 58 P types based on serological and genetic characterization of outer capsid proteins VP7 (G-type) and VP4 (P-type). At least 16 G and 19 P types are recognized in humans (8-10). With the increasing availability of rotavirus whole genome sequences, rotaviruses are now classified into genome constellations based on sequence similarities of all 11 dsRNA segments (9, 11). Three genome constellations have been identified to date: Wa-like, DS-1 like and AU-1 like, with the first two being the most common (9, 11, 12). Genome-based classification offers additional information on genome diversity, possible zoonotic transmission, segment reassortment, antigenic drift and strain evolution and is becoming increasingly adopted; however, VP7 and VP4 genotyping remains widely used in most laboratories (12).

Six genotypes of group A rotavirus: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] account for >90% of the rotavirus infections worldwide (13). Continued evolution of the rotavirus genome, due to an error-prone RNA polymerase, may give rise to unpredictable strain predominance which varies greatly by year and by geographic location (13, 14). In recent years, uncommon genotypes have emerged and spread globally including G9P[8], G3P[8], G12P[8] and vaccine-derived G1P[8] (15-19).

Rotavirus infections manifest as a spectrum of symptoms ranging from asymptomatic to life-threatening (8). Previous studies have presented conflicting data on the association of rotavirus genotypes/strains and illness severity. For example, studies from Latin America, India, Iran, Cambodia and the UK found that children with emerging G9 and G12 strain infections had more severe acute gastroenteritis (AGE) than those with other genotypes (18, 20). However, the association of clinical severity with genotype was not evident in studies conducted in France, US, Korea and Italy (21, 22).

Rotavirus vaccination is the best strategy to reduce the burden of severe and life-threatening rotavirus gastroenteritis. Rotarix is monovalent, live-attenuated vaccine of a human G1P[8] rotavirus strain; RotaTeq® is a live-attenuated vaccine of five human-bovine reassortant viruses displaying antigens of G1, G2, G3, G4 and P[8] strains (23). Although the implementation of vaccines has reduced the rotavirus burden, increased vaccine-induced immune pressure against wild-type rotavirus strains may select for immune escape mutants or antigenically distinct strains (24). Therefore, post-vaccine-implementation surveillance of circulating rotavirus genotypes is vital to monitor changes in transmission patterns, identification of vaccine-induced novel heterotypic genotypes (or neutralization escape mutants), and discovery of potentially pathogenic vaccine related strains (25).

In the province of Alberta, Canada, the monovalent rotavirus vaccine (RV1) Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) was included in the routine universal childhood immunization schedule in June 2015 and was replaced by the pentavalent RotaTeq® in May 2018, and was again introduced from May 2021 onward (26). However, the impact of RV1 vaccination on the emergence of novel rotavirus genotypes, the presence of RV1 derived strains, and genotype specific clinical characteristics in children with rotavirus AGE are not well understood. To fill this knowledge gap, we examined the genotype distribution and clinical characteristics of rotavirus illness in children with AGE in four consecutive seasons between 2014 and 2018 before and after rotavirus vaccine introduction in Alberta.

## Methods:

Our primary aim was to examine the disease incidence of rotavirus in Alberta and characterize seasonality and genotype diversity of circulating rotavirus, including AGE attributed to RV1 derived strains detected

in our cohort. Secondary objectives include the stratification of rotavirus infection by participants' age, vaccination status, and clinical characteristics of rotavirus gastroenteritis and common rotavirus genotypes to determine the clinical features associated with emerging strains.

### *Study participants and specimen acquisition*

Children < 18 years of age with AGE were recruited consecutively by the Alberta Provincial Pediatric EnTeric Infection TEam (APPETITE) according to the study protocol (27). Caregiver consent along with participant assent were obtained as appropriate. Children with AGE, defined as [?]3 episodes of vomiting or diarrhea in the preceding 24 hours and symptom duration <7 days, were recruited through two pediatric emergency departments (EDs) and a telephone nursing advice line in Alberta between December 2014 and August 2018. Ethics approval was provided by the University of Alberta and University of Calgary research ethics boards (Pro00050790 and REB14-1122).

Rectal swabs and stool specimens were collected from eligible children. FLOQSwabs™ (Copan Italia, Brescia, Italy) were inserted into the rectum and rotated 360o then placed into sterile sheathes. Stool specimens were collected in Fecal Collectors (V302-F, Starplex Scientific, ON, Canada). Specimens were transported at 4degC to the testing laboratory within 24h of collection and frozen immediately at -70degC until testing was performed. Specimens were analyzed within a week of arrival in the laboratory.

### *Molecular detection of rotavirus*

Details of nucleic acid extractions, reverse transcription, and rotavirus molecular detection were described previously (19). Briefly, total nucleic acid was extracted from 30% rectal swab suspension or 10% stool suspension, prepared using NucliSENS(r) lysis buffer (BioMerieux, Laval, QC, Canada) by NucliSENS(r) easyMAG(r) (BioMerieux, Laval, QC, Canada) according to manufacturer's instructions. All resultant nucleic acid extracts were immediately stored at -70degC until testing. cDNA was synthesized from viral RNA using SuperScript™ II reverse transcriptase (Invitrogen, MA, USA). Group A rotaviruses in stool and swabs were identified by an in-house reverse transcription real-time PCR (RT-qPCR) assay, the Gastroenteritis Viral Panel (GVP), and a multi-target bead-based molecular assay, the Luminex Gastrointestinal Pathogen Panel (GPP) (Luminex Molecular Diagnostics, ON, Canada), as described previously (28).

### *VP7 and VP4 genotyping approach*

Nucleic acid was extracted from stool or rectal swab specimens (19). Specimens with the lowest RT-qPCR Ct value (corresponding to higher viral load) were subsequently subjected to VP7/VP4 genotyping using conventional RT-PCR and electrophoretotyping with genotypes being determined by banding pattern (29) with updated G12 primers (30) (Supplemental Methods). A subset of specimens that could not be genotyped due to polymorphisms at the primer binding sites or low viral load, were subjected to an alternative sequence-based method of G/P genotyping specifically designed for low viral load samples (15) (Supplemental Methods). The sequences obtained were analyzed using MEGAv7 software and genotypes were aligned to reference sequences available in GenBank (31).

### *Identification of RV1 strains*

cDNA of all AGE cases that tested positive for rotavirus in the primary study was retested using a validated RV1 qPCR assay to differentiate RV1 vaccine strains from wild type rotavirus (19). RV1 strains were identified by harbouring an NSP2 gene segment of vaccine origin.

### *Whole genome sequencing*

To fully understand the genetic relatedness of RV1 derived strains to the original RV1 strain, complete genome sequencing was performed on 20 RV1 derived strains and 24 wild type G1P[8] strains selected on the basis of specimen availability and a high viral load (i.e., Ct values<30). Rotavirus RNA extraction, library preparation and sequencing were performed according to an unpublished rotavirus whole-genome sequencing protocol generously provided by the US Centers for Disease Control and Prevention (CDC) with the following modifications: the use of the most recent version of New England Biolabs (NEBNext Ultra II) RNA library

preparation kit for Illumina and Illumina MiSeq kit, and an updated extraction method. Briefly, 30% stool filtrates were produced by diluting 300  $\mu$ L of liquid stool specimen or 300 mg of solid stool specimen in 700  $\mu$ L of PBS, vortex-homogenized, and centrifuged for 15 mins at 14,000 rcf. Finally, samples were filtered through Durapore-PVDF Ultrafree-MC Centrifugal Filters with 0.65  $\mu$ m pore size (EMD-Millipore, Canada). Viral dsRNA was extracted using QIAamp<sup>®</sup> Viral RNA Mini Kit (Cat No./ID: 52904, QIAGEN, USA) following manufacturer’s recommendation. The quality of purified dsRNA was assessed by rotavirus RT-qPCR. Five microliters of dsRNA in the 10-100 ng concentration range, as measured by Qubit RNA HS Assay Kit (Q32852, ThermoFisher Scientific, USA) was used to generate cDNA libraries with NEBNext<sup>(r)</sup> Ultra II RNA Library Prep Kit for Illumina<sup>(r)</sup> (E7770S, New England Biolabs, USA) and indexed using NEBNext<sup>(r)</sup> Multiplex Oligos for Illumina<sup>(r)</sup> (96 Unique Dual Index Primer Pairs) (E6440S, New England Biolabs, USA) following manufacturer’s protocols. cDNA libraries were quantified using Qubit dsDNA HS Assay Kit (Q32851, ThermoFisher Scientific, USA). The average size of the libraries was visualized on Agilent TapeStation D4200 with High Sensitivity DNA D1000 ScreenTape analysis kit (both from Agilent, USA). Rotavirus RT-qPCR was used again to detect rotavirus insert in the cDNA libraries. Selected libraries were quantified using NEBNext<sup>(r)</sup> Library Quantification kit for Illumina<sup>(r)</sup> (E7630S, New England Biolabs, USA) on ABI 7500 real-time PCR instrument (ThermoFisher, USA). Libraries were normalized to 4 nM concentration and sequenced on Illumina MiSeq platform at the Bacterial Typing Unit of the Alberta Public Health Laboratory Edmonton (Alberta Precision Laboratories) with Illumina MiSeq Kit V3 (150-cycle, cat MS-102-3001; Illumina, USA).

#### *RV1 genome constellation analysis and phylogenetics*

Illumina short read sequences were quality filtered using Trimmomatic v0.39 software (32). Quality-filtered reads were classified against all rotavirus sequence information from NCBI (downloaded 2019-12-02; n=76,138) with Kraken 2.0.8-beta (33). Consensus genomes were constructed by first finding the closest matching segments against all full-length rotavirus segments from NCBI (n=54,005 sequences from all files downloaded above on 2019-12-02) using Mash 2.2.2 screen (34). The sequence with the closest mash-distance was used as a reference, where the Kraken2-classified sequences were mapped using BWA MEM 0.7.17-r1188 (arXiv:1303.3997v2). Consensus sequences for each segment were generated using SAMtools v1.9 (35) and iVar v1.0 (36). Rotavirus genome depth and coverage were plotted using SAMtools, R 3.6.1 (R Core Team; <https://www.R-project.org>), ggplot2 v3.2.1 (Wickham; 2016; <https://ggplot2.tidyverse.org>) and viridis v0.5.1. RV1 whole genome sequences from the four specimens were uploaded to the Viral Pathogen Resource (ViPR) website to determine the genome constellation of the strains and results were compared to RV1 constellation (accessed in May 2022, now available at the Bacterial and Viral Bioinformatics Resource Center <https://www.bv-brc.org>).

#### *Patient clinical characteristics, gastroenteritis severity measure and RV1 vaccine status*

Given that G1P[8] was the most prevalent genotype worldwide before the widespread use of vaccines, we compared the clinical characteristics of participants whose stool contained the emergent G12P[8], RV1, G2P[4] or G9P[8] strains to that of participants infected by the wild type G1P[8] rotavirus. The description of illness collected included the presence of fever, duration of diarrhea and vomiting, modified Vesikari score (MVS) (37, 38), and presence of dehydration measured by clinical dehydration scores (39). MVS score severity was categorized as mild (0-8), moderate (9-10), and severe ([?] 11). The analysis also included age, sex, co-detected pathogens, and the participant’s rotavirus vaccination status which was obtained from Alberta Health’s Imm/ARI database.

#### *Statistical analysis*

Annual rotavirus season was defined as a 12-month period from August to July to provide a 2-month period after the June 2015 implementation of rotavirus vaccine to account for effects of the vaccine program. Continuous variables were summarized using medians (IQR) and compared between groups using the Mann-Whitney U test or Kruskal-Wallis test, as appropriate. We summarized categorical variables using proportions and compared groups using the Chi-square test of association or Fisher’s Exact test, as appropri-

ate. Statistical significance was defined by a two-tailed  $P$ -value  $<0.05$ . Descriptive statistics were computed using GraphPad Prism v8. Between group comparisons were performed using SPSS 25.0 (40).

## Results :

### *Molecular depiction of rotavirus detected*

Rotavirus was detected in 17.2% (577/3347) of the 3374 participants who submitted specimens (Figure 1). G1P[8] was the most prevalent genotype (25.8%, 149/574), followed by G12P[8] (21.5% 124/577), G9P[8] (20.6%, 119/577) and G2P[4] (14.2%, 82/577) genotypes. Prior to the introduction of RV1 in Alberta (i.e., 2014-15), G9P[8] predominated; however, following RV1 introduction, G1P[8], G2P[4] and G12P[8] were the primary genotypes during the 2015-16, 2016-17 and 2017-18 seasons, respectively (Table 1). Of note, the RV1 derived strain, was detected during the three seasons post RV1 implementation but not prior. Unusual strains of common human G2 or G9 types combined with potential zoonotic P[6] or P[9] types and two mixed infections were also identified.

Rotavirus was detected year-round with peaks observed between March and May (Figure 2). Prior to RV1 introduction in 2014-15, almost 60% of the total number of rotavirus AGE cases occurred during its seasonal peak. This was reduced to below 40% the first year, below 20% the second year and back to below 40% during the third year after vaccination initiation.

### *Genome constellation analysis of RV1 strains recovered from children with AGE*

The nucleotide sequences of 11 gene segments were successfully obtained in four RV1 containing specimens with relatively high viral loads. Due to low titers, the Illumina sequencing wasn't sensitive enough to recover entire genomes of the other RV1 strains. All four RV1 isolates had the Wa constellation of G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 which is consistent with the original RV1 genome composition. The 24 wild type G1P[8] strains yielded Wa constellation as expected.

### *Epidemiological characterizations and clinical severity of rotavirus infections*

Children with RV1 were significantly younger [median age 3.2 (2.4, 3.8) vs 20.8 (14.6, 32.8) months old, ( $p<0.0001$ )], more likely to be female (56.8% vs 38.9%,  $P =0.039$ ), and to have mild symptoms and overall disease severity than those with wildtype G1P[8] (Table 2). Of all genotypes, RV1 was identified most frequently in children under 3 months of age (~10% vs. ~1% for all others) (Figure 3). Children with the emergent G12P[8] genotype were older (24-59 month) and more often female (53.2% vs 38.9%,  $P =0.02$ ) than those with wildtype G1P[8] (Table 2; Figure 3). However, there was no increase in the proportion of participants who were older during the 2017-2018 epidemic season when G12P[8] caused the majority of rotavirus infections (Figure S1). Children with G2P[4] were less likely to be hospitalized (4.0% vs 15.1%;  $P =0.01$ ) and had lower MVS scores [14 (11, 16) vs 15 (12, 17);  $P =0.03$ ] than those with G1P[8](Table S1). With the exception of RV1 infection, children with major rotavirus genotypes had similar clinical symptoms, management, and disease severity scores (Table 2 and S1). Furthermore, co-infection with other gastroenteritis viruses, enteric bacteria, or parasites in these children did not result in different clinical characteristics (Table 2).

In unvaccinated children, AGE was most commonly due to the four predominant genotypes each season: G1P[8], G2P[4], G9P[8], and G12P[8] but not RV1 (Figure 4). RV1 was the predominant strain, detected in more than 50% of children with AGE who had received 1 or 2 RV1 doses (Fisher's exact test,  $P <0.001$ ).

## Discussion :

In the months preceding RV1 introduction, rotavirus G9P[8] was the predominant genotype with a high percentage of G1P[8] strains also detected. Following the implementation of a publicly funded RV1 vaccination program in Alberta with greater than 80% coverage(41), we observed a large reduction in the percentage of AGE due to rotavirus infections with the peak of activity diminishing during the 2015 to 2018 study years (Figure 2). While the circulating rotavirus genotypes remained mostly unchanged, G9P[8] was no longer the

prevailing strain with the homotypic G1P[8], heterotypic G2P[4], and the emerging G12P[8] strains becoming predominant.

Consistent with previous reports, we found that strain predominance varies from year to year. However, more comprehensive multiyear data are needed to ascertain whether a delayed detection peak, a shortened duration of the peak, or a shift from annual to biennial epidemics is observed locally post vaccination introduction as documented in the US (42). Controversy exists related to the long-term effect of vaccination on the circulating genotype diversity (43). Whether the shift in rotavirus genotype distribution in our study is due to natural fluctuations as part of the inherent evolution of the virus, or to the emergence of new vaccine-escape mutants due to selection pressure from RV1 remains to be clarified.

The genotype constellation of the RV1 isolates found in our cohort closely resemble the original RV1 virus. We did not identify any vaccine and wild type rotavirus reassortments in the small number (n=4) of RV1 strains analyzed. Improved sensitivity of Illumina sequencing for low viral load rotavirus samples is needed for future studies. RV1 strains were associated with very mild AGE in infants under three months and were detected in children who had received a single vaccine dose (19). We could not differentiate between symptomatic infection by RV1 and post-vaccine shedding of RV1 and incidental gastroenteritis symptoms in these children.

G1P[8] was the predominant genotype reported previously in a multisite genotype study, including Alberta, conducted under the Canadian Immunization Monitoring Program, Active (IMPACT) (unpublished data) in 2014 and 2015. In this study, we found that G9P[8] had the highest prevalence in the short eight-month surveillance season of 2014-2015, comprising 31.9% of strains. The G9P[8] genotype was first reported in the 1980s and has been widespread globally since the mid-1990s, circulating as a minor type or predominant strain, accounting for >70% of all rotavirus cases (17, 18).

Increases in G2P[4] prevalence have been reported in countries and regions with consistent RV1 vaccine utilization (44), raising the question of whether RV1 offers limited cross-protection against the heterotypic G2P[4] strains since the genetic backbone (i.e. genotype constellation) of the two (Wa-like vs DS1-like) are different. Although we detected a transient increase in G2P[4] prevalence in the second year following RV1 introduction, this must be interpreted cautiously as the heterotypic G2 is often associated with cyclic re-emergence during 10-year periods (44). As such, it is not clear if the rise in G2P[4] detection was due to vaccine selection pressure or simply reflecting the natural oscillation of this genotype. A review of G2P[4] strain evolution reported that alterations in G2P[4] distribution occur commonly in countries that use RV1, RV5 or mixed vaccination strategies as well as in countries without routine rotavirus vaccination (44). Our data, support the global data (44) that there has not been a surge of G2P[4] driven by vaccine selection pressure.

Strains of the G12 genotype were first described in infants with AGE in southeast Asia in 1987 and 1998 (45, 46). Since then, the G12P[8] genotype has been increasingly detected around the globe and gained predominance as the sixth epidemic strain during the past few years (47, 48). In Alberta, G12P[8] strains first emerged in 2012 and became predominant in 2018 according to provincial viral AGE surveillance data (unpublished data). We found that G12P[8] predominantly infected older children and was the most common strain in fully vaccinated children. This suggests that G12P[8] may have unique epitopes that evade host immune responses generated from vaccination or prior infection by another rotavirus genotype. Thus, RV1 vaccine may provide insufficient cross-protection against this emergent strain. Indeed, multiple antigenic mismatches were identified in the VP7 (G protein) of the predominant G12P[8] and the vaccine strains in the US (47). Consistent with our results, G12P[8] infection was more common among partially or fully vaccinated infants than other rotavirus genotypes. However, an overall reduction in rotavirus activity occurred in 2017-2018 when G12P[8] predominated suggesting good protection of RV1 against rotavirus AGE. Additional studies with longer surveillance periods and larger sample sizes are warranted to understand vaccine effectiveness against G12P[8]-specific rotavirus AGE.

Knowledge of the association between rotavirus genotype diversity and AGE clinical severity is limited.

Previous studies have presented contradictory findings on this topic (18, 20-22). In our study, children with RV1 detected in their stool specimens had less severe AGE episodes, consistent with symptoms due to the live attenuated vaccine virus. We also found that children with G2P[4] infections were less likely to require hospitalization than those with G1P[8]. While the median MVS score for children with G2P[4] infections was in the severe range and clinical symptoms and other severity measures were comparable with those of G1P[8], the median total MVS score was one point lower than that of total rotavirus cases regardless of genotype. Our data suggest that other than RV1 related G1P[8] isolates, the clinical severity of different rotavirus genotypes did not differ significantly. Wild type rotavirus of all genotypes detected were found to cause severe AGE in young children thus emphasizes the need for rotavirus vaccines that protects against all genotypes.

A limitation of our study is that the assay utilized was unable to detect the new equine-like G3P[8] strains. Thus, future studies with updated primer sets (49) are needed to obtain a more complete picture of circulating rotavirus diversity. Moreover, as the rotavirus genotype prevalence fluctuates naturally over a period that varies between 3 and 11 years (50), a longer surveillance period that includes additional pre- and post-vaccine years is needed to fully understand the impact of rotavirus vaccination on local rotavirus diversity.

In summary, our study revealed dynamic changes in rotavirus genotype prevalence before and after RV1 introduction into Alberta's routine immunization program: G9P[8], G1P[8], G2P[4] and G12P[8] predominated consecutively each season. This emphasizes the need for ongoing monitoring of vaccine effectiveness against all genotypes as most continue to be associated with severe AGE in children. Continued rotavirus surveillance and detailed whole genome characterization of strains could be employed to determine the changes in epidemiology and distribution of rotavirus genotypes and to assess the potential impact of vaccination selection pressure on rotavirus evolution in the post vaccination era.

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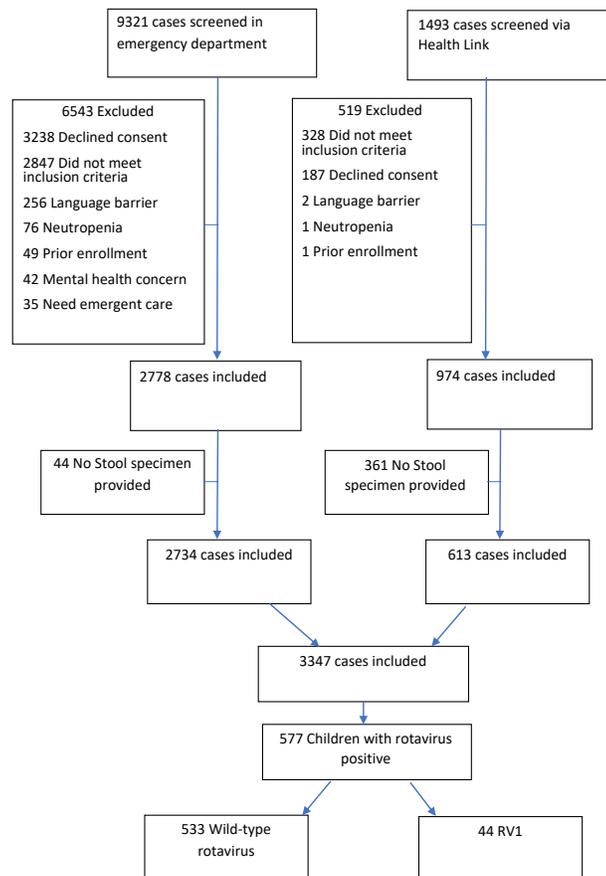


Figure 1. Study flowchart

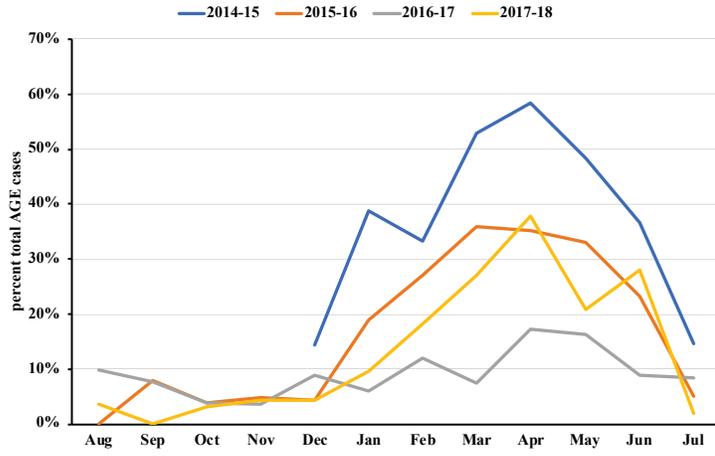


Figure 2. Monthly frequency of rotavirus positives during four consecutive seasons 2014-2018

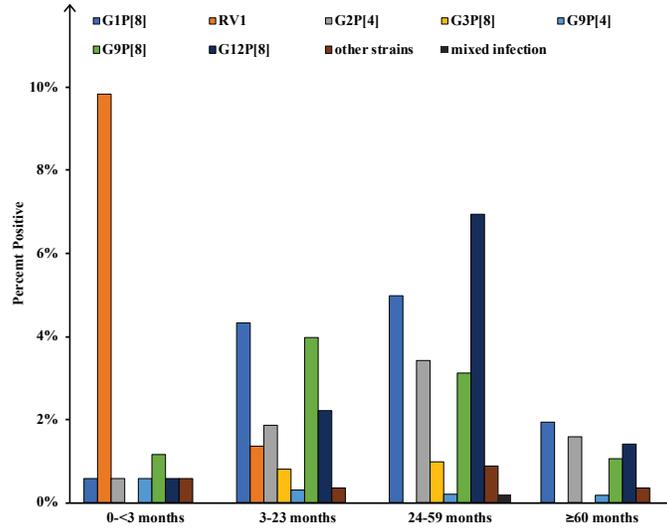


Figure 3. Rotavirus genotype distribution across age groups. Number of participants per each group is as follows: 0-<3 months, n/N=23/173; 3-23 months, n/N=302/1992; 24-59 months, n/N=212/1024; ≥60 months, n/N=37/565. N is the total number of AGE children enrolled in each age group.

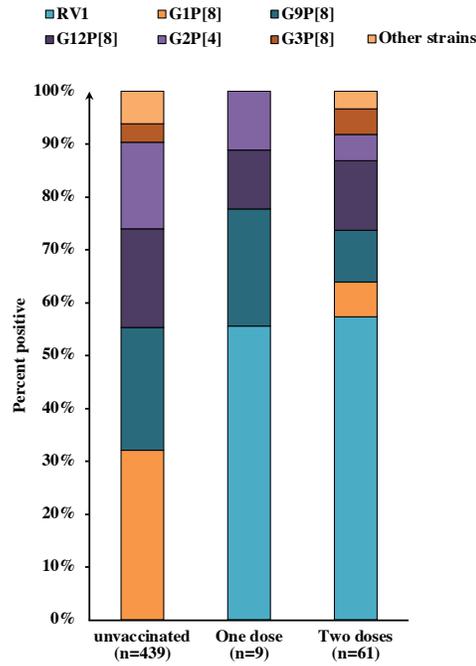


Figure 4. Rotavirus genotype distribution in children with varying vaccine status.

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