Rotavirus Strain Surveillance in Estonia After Introduction of Rotavirus Universal Mass Vaccination

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Background: Estonia implemented the rotavirus (RV) vaccine into its national immunization program in July 2014. We aimed to determine circulating RV genotypes and the clinical profile by genotypes from February 1, 2015, to August 30, 2016, among children 0–18 years hospitalized due to rotavirus gastroenteritis (RVGE).

Methods: During an observational study in 7 Estonian hospitals, we determined the RV genotypes in stool samples of RVGE patients who met predetermined criteria. Shannon's diversity index (H′) and Simpson's index (D) was used to evaluate genotype diversity by season and age and to compare prevaccine period data (2007–2008) for children 0–4 years of age (n = 77) to corresponding data from the postvaccine period (2015–2016, n = 346). The Vesikari Clinical Severity Scoring System was used for clinical profile evaluation.

Results: Stool samples of 479 RVGE patients were genotyped. Seventy-seven percent of RVGE infections were caused by G4P[8] (n = 150, 31%), G1P[8] (n = 100, 21%), G9P[8] (n = 79, 16%), G2P[4] (n = 23, 5%), G4P[4] (n = 17, 4%). The prevailing genotypes varied seasonally. Diversity increased during the postvaccine period among age groups 0–4: H′ 1.42 (95% CI: 1.2–1.7) in the prevaccine era versus 1.8 (95% CI: 1.7–2) in the postvaccine era ($P = 0.008$), and D 0.6 (95% CI: 0.5–0.7) versus 0.78 (0.75–0.80) ($P = 0.01$), respectively. The off-season period presented higher diversity compared with in-seasons. G2P[8], G1P[8], G4P[4], G9P[8], and G8P[8] presented with a different clinical profile compared with others.

Conclusion: Since the introduction of universal mass vaccination in Estonia, the circulating RV genotypes have changed compared with those reported in the prevaccine era. Our study adds to knowledge about RV genotype distribution in Europe and expected dynamics after RV universal mass vaccination and provides insight on the clinical profile of prevailing genotypes.

Key Words: rotavirus, genotypes, vaccine, diversity, Estonia

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Rotavirus (RV) has been the leading cause of acute gastroenteritis (AGE) among children under the age of 5 years with an estimated 3.6 million cases, 700,000 outpatient visits, 87,000 hospitalizations, and 231 deaths annually in Europe.[1] With the implementation of universal RV mass vaccination (UMV) in several countries since 2006, the burden of rotavirus gastroenteritis (RVGE) has started to decline. However, further knowledge of circulating RV genotypes during the vaccine era is needed to achieve progressive decrease of the global RVGE burden.[2,3]

Rotavirus has 10 RV genogroups (A through J), with genogroup A responsible for most human disease.[4,5] Of over 60 G-genotype/P-genotype combinations of A genogroup rotaviruses infecting humans, the following 6 combinations have been most prevalent worldwide during RV in-seasons: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8].[3,6–8] Greater genotype diversity and higher proportional representation of nontypeable, mixed, and less common genotypes have been reported during off-season periods compared with the in-season time period.[9]

RV1 (Rotarix, GlaxoSmithKline, Belgium), a single-strain G1P[8] human vaccine given as 2 doses, and RV5 (RotaTeq, Merck and Co. Inc., United States), a pentavalent G1, G2, G3, G4, and P[8] human-bovine WC3 reassortant vaccine given as 3 doses, were introduced in 2006. Although postvaccine licensure surveillance has not shown a consistent global pattern indicative of selection pressure resulting from RV vaccines, an increase in genotype diversity among symptomatic cases and transient predominance of less common strains have been reported.[7,8,10,11] Whether emerging variation is due to vaccine pressure or natural fluctuation of RV remains to be determined, as there have been reports of changes in genotype distribution during the postvaccine era regardless of RV universal mass vaccination (UMV) presence. For example, compared with previous in-seasons, the European Rotavirus Network (EuroRotaNet) reported changes in RV genotype distribution in 14 European countries during the 2015/2016 RV in-season regardless of RV UMV presence, with a shift toward G2P[4] and G9P[8] strains predominating.[8]

During the prevaccine era in 2007–2008 in Estonia, the dominant genotype was G2P[4] (34.7%), which caused significantly more cases than G4P[8] (12.9%), G1P[8] or G9P[8] (both 4.0%), and G3P[8] (1.6%).[12] In other European countries during the same timeframe, the prevailing genotypes were most commonly G1P[8] or G9P[8].[6,12] The only countries without G1P[8] or G9P[8] dominance were Austria and Belgium.[13]

RV1 and RV5 became available for purchase in Estonia in 2008, and vaccine coverage among vaccine-eligible children increased each year from 2% in 2008 to 15% in 2013.[14] RV UMV was initiated in July 2014, with RV5 being replaced by RV1 in October 2015 because of national procurement. Vaccination coverage of 65.6% and 86.8% was achieved by the end of 2015 and 2016, respectively.[15,16]

To provide knowledge of RV genotype distribution after RV UMV implementation, we aimed to describe circulating RV genotypes during the first 2 consecutive RV in-seasons after RV UMV initiation among children with 0–18 years of age who were hospitalized due to rotavirus gastroenteritis and to describe the clinical profile by RV genotypes.

METHODS

We conducted a prospective observational multicenter study from February 1, 2015, to August 30, 2016, in 7 hospitals (2 regional hospitals: Tartu University Hospital and Tallinn Children's Hospital; 2 central hospitals: West-Tallinn Central Hospital and Pärnu Hospital; 3 general hospitals: Ida-Viru Hospital, Kuressaare Hospital, and South-Estonian Hospital) that cover 80% of pediatric hospitalizations in Estonia. All children with 0–18 years of age presenting to the emergency department with acute gastroenteritis (AGE) were screened for eligibility by the study doctor on duty. AGE was defined as 3 or more loose stools and at least 1 forceful vomiting episode within the last 24 hours that were unexplained by any other medical condition.

At hospitalization, demographic data (age, gender, nationality, RV vaccination history) and disease severity, categorized according to the Vesikari Clinical Severity Scoring System (VCSS) as mild <7, moderate 7–10, and severe 11–20 points,[17] were recorded at study entry to the web-based study database. Patients' rotavirus status was determined by the RV antigen test performed routinely in hospital laboratories.

Sample Collection and Identification

A stool sample was collected at the very first opportunity when a bowel movement occurred at admission or thereafter until discharge. No further measures were taken to obtain a stool sample after discharge. Samples were stored at −20°C until sent to the medical laboratory of Synalb Eesti OÜ within 24 hours and stored at −80°C until RNA extraction. The maximum time from sample collection to RNA extraction was 4 weeks.

RNA Extraction and Genotyping

In the laboratory, RNA was extracted with the Roche MagNAPure 96 Instrument according to the manufacturer's protocol, Pathogen 200.[18] Extracted RNA samples were stored at −80°C until sent to the Department of Microbiology at the Institute of Biomedicine and Translational Medicine, University of Tartu for RV genotyping.

Samples of patients who had documented RVGE diagnosis at discharge, based on commercial RV antigen detection tests done at the laboratories of the study hospitals, were genotyped using seminested reverse-transcription PCR according to the WHO "Manual of RV detection and characterization methods" (2009) with primers recommended for Europe in Figure (Supplemental Digital Content 1, http://links.lww.com/INF/E2761). Genotypes were determined by evaluating agarose-gel electrophoresis results of PCR products according to the WHO 2009 Manual.[19]

Data Analysis

Patient characteristics were described by relative frequencies for categorical data and medians and interquartile ranges (IQR) for continuous data.

The Kruskal-Wallis test was used to determine differences by genotype in time to hospitalization, length of hospital stay, number of diarrhea episodes, and number of vomiting episodes and Fisher's exact test to compare gender distributions and the proportion of vomiting, receiving intravenous rehydration and being vaccinated between genotypes. Analysis of variance (ANOVA) was used to analyze the differences of the Vesikari mean scores by genotype.

The start of RV season was defined according to the European Centre for Disease Prevention and Control (ECDC) criteria as the first of 2 consecutive weeks during which the percentage of specimens taken from patients with AGE that tested positive for RV exceeded 10%, and the end of RV season was defined as the last of 2 consecutive weeks during which the percentage of specimens positive for RV was less than 10%. Off-season was considered to be the time period between in-seasons according to the ECDC definition.[20]

Only genotypes making up more than 1% of cases during both RV in-seasons were included in statistical tests comparing genotypes.

Genotype diversity by RV in-season, off-season versus RV in-seasons, prevaccine period (2007–2008) children with 0–4 years of age (n = 77) versus study period children with 0–4 years of age (n = 346) and by age was compared using Simpson's index of diversity and Shannon's index. Bootstrap bias-corrected 95% confidence intervals were calculated to diversity indices, and *P* values were calculated based on bootstrapped samples.[21] When comparing diversity indices in different age groups, *P* values were corrected for multiple testing using the Holm-Bonferroni method. Data of mixed and nontypeable samples were excluded from diversity calculations.

In all analyses, we defined $P < 0.05$ as statistically significant.

All statistical analyses were performed with Stata/IC 14.2.

Ethical Considerations

The Research Ethics Committee of the University of Tartu reviewed and approved the study protocol. Parents or guardians signed informed consent forms before study entry. Informed assent forms were also signed by children with 7–18 years of age.

RESULTS

RVGE Patients

During the study period, 2249 patients with 0–18 years of age with AGE were hospitalized. The median age of hospitalized children was 2 (IQR, 1–5) years. RVGE diagnosis at discharge was documented in 567 (25%) of cases: 402 (70.9%) in the 2015 RV in-season (starting at week 9 and ending at week 35); 125 (22.1%) during the 2016 RV in-season (starting at week 9 and ending at week 36 when the study was stopped); and 40 (7.1%) patients in the off-season (lasting from week 36 in 2015 to week 8 in 2016). Stool samples were available for 486 (86%) RVGE patients, and 81 (14%) did not have a bowel movement during their hospital stay. Seven samples (1.4%) were excluded from further analysis due to concomitant laboratory diagnosis of bacterial enterocolitis.

Detailed characteristics of patients with genotyped samples are presented in Table 1.

According to the Estonian Health Insurance Fund, the only health insurance provider in Estonia whose database covers the demographics, ICD-10 codes and treatment facilities of all patients of Estonia, 83% (n = 898) of all RVGE patients were hospitalized into our study hospitals during the study period. We were able to recruit 567 (63%) and obtain 486 (86%) stool samples for genotyping. As we included hospitals from 7 different regions of Estonia, all areas are represented. Overall, our study results represent 45% of RVGE patients in Estonia in 2015–2016.

Genotype Distribution Post UMV

During the study period, the majority of RVGE infections (77%) were caused by genotypes G4P[8] (n = 150, 31%), G1P[8]

TABLE 1. Characteristics of RVGE Patients With Genotyped Samples

	RV In-season 2015	RV In-season 2016	Off season*	Total
RVGE patients with genotyped samples, n	336	108	35	479
Median age, n (IQR)	2 (1–4)	3 (2–5)	3 (1–5)	2 (1–4)
Male, n (%)	179 (53.3)	50 (46.3)	20 (57.1)	249 (52)
Age group, n (%)				
<1	23 (6.8)	7 (6.5)	1 (2.9)	31 (6.5)
1–4	251 (74.7)	71 (65.7)	23 (65.7)	345 (72)
5–9	60 (17.9)	27 (25)	9 (25.7)	96 (20)
10–14	2 (0.6)	3 (2.8)	2 (5.7)	7 (1.5)
15–18	0 (0)	0	0 (0)	0 (0)
Median time to hospitalization, d (IQR)	1 (0–3)	1 (0–2)	1 (1–3)	1 (0–3)
Median length of hospitalization, d (IQR)	3 (2–4)	2 (2–3)	3 (2–4)	3 (2–4)
VCSS† n (%)				
Moderate	14 (5)	5 (4.8)	2 (6.9)	21 (5.1)
Severe	266 (95)	100 (95.2)	27 (93.1)	393 (94.9)
Vaccination status‡, n (%)				
Yes	13 (3.9)	13 (12)	4 (11.4)	30 (6.3)
No	317 (94.3)	93 (86.1)	31 (88.6)	441 (92.1)
Not known	6 (1.8)	2 (1.9)	0 (0)	8 (1.7)

*Off-season: weeks during which the percentage of patients with RV diagnosis among hospitalized AGE patients remained below 10%.

†VCSS (mild <7, moderate 7–10, severe ≥11 points; maximum score: 20 points) of those who had all parameters documented.

‡Vaccination status: patient has received 1 or more RV vaccine dose.

AGE indicates acute gastroenteritis; IQR, interquartile range; RV, rotavirus; RVGE, rotavirus gastroenteritis; VCSS: Vesikari Clinical Severity Scoring System.

(n=101, 21%), G9P[8] (n=79, 16%), and G2P[4] (n=23, 5%). Only 15 (3%) samples were negative or partially typed (only G or only P genotype). Variation in genotype distribution was seen during RV in-seasons compared with the off-season period (Table 2). In the 2015 in-season, the most common genotypes were G4P[8], G1P[8], G9P[8], and G2P[4]. In 2016, genotypes G9P[8], G4P[4], G9P[4], and G2P[8] prevailed. The overall proportion of genotypes and mixed infections causing less than 1% of cases during both RV in-seasons increased from 4% (n=13) in 2015 to 9% (n=10) in 2016 and were the cause of the majority (34.3%) of RVGE hospitalizations during the off-season (Table 2).

Genotype Diversity

During the study period, we identified 26 different RV genotypes.

According to both diversity indices, genotype diversity was significantly increased during the off-season period compared with RV in-seasons: H′ 1.9 (95% CI: 1.8–2.0) during in-seasons 2015–2016 versus 2.4 (95% CI: 2.3–2.6) during the off-season ($P=0.008$) and D: 0.78 (95% CI: 0.75–0.80) versus 0.9 (95% CI: 0.81–0.91) ($P=0.038$), respectively.

Shannon's diversity index, which in contrast to Simpson's focuses on the presence of different genotypes in a period rather

TABLE 2. RV Genotypes in 2015 and 2016, Post-UMV in Estonia

Genotype	RV In-season 2015		RV In-season 2016		Off-season§		Total
Common genotypes	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n (%)
G4P[8]	144	43 (37.7–48.2)	4	4 (1.4–9.5)	2	6 (1.4–20.6)	150 (31)
G1P[8]	97	29 (24.3–34)	2	2 (0.5–7.2)	2	6 (1.4–20.6)	101 (21)
G9P[8]	26	8 (5.3–11.1)	43	40 (31–49.4)	10	29 (16–45.8)	79 (16)
G2P[4]	22	7 (4.3–9.8)	1	1 (0.1–6.4)	0	NA	23 (5)
G12P[8]	6	2 (0.8–3.9)	2	2 (0.5–7.2)	2	6 (1.4–20.6)	10 (2)
Less common genotypes							
G9P[4]	0	NA	11	10 (5.7–17.5)	2	6 (1.4–20.6)	13 (3)
G2P[8]	4	1 (0.5–3.1)	8	7 (3.7–14.1)	2	6 (1.4–20.6)	14 (3)
G4P[4]	2	1 (0.2–2.4)	13	12 (7.1–19.7)	2	6 (1.4–20.6)	17 (4)
G8P[8]	4	1 (0.5–3.1)	1	1 (0.1–6.4)	0	NA	5 (1)
G2P[6]	1	0 (0.04–2.1)	3	3 (0.9–8.3)	0	NA	4 (1)
G9P[9]	1	0 (0.04–2.1)	2	2 (0.5–7.2)	0	NA	3 (1)
Mixed*	6	2 (0.8–3.9)	4	4 (1.4–9.5)	0	NA	10 (2)
Other†	13	4 (2.3–6.6)	10	9 (5–16.4)	12	34 (20.4–51.5)	35 (7)
Nontypeable‡	10	3 (1.6–5.5)	4	4 (1.4–9.5)	1	3 (0.4–18.2)	15 (3)
Total	336	100	108	100	35	100	479 (100)

*Mixed: more than 1 genotype found in an individual sample

†Other: genotypes and mixed infections making up less than 1% cases during both RV seasons

‡Nontypeable: negative or partially typed strains (only G or only P)

§Off-season: weeks during which the percentage of patients with RV diagnosis among hospitalized AGE patients remained below 10%.

CI indicates confidence intervals; NA, not applicable; RV, rotavirus; UMV, universal RV mass vaccination.

than relative abundance, also showed a significant increase in RV genotype diversity in the second RV in-season post UMV in 2016 compared with the 2015 RV in-season: H′ 1.5 (95% CI: 1.4–1.6) in 2015 versus 1.95 (95% CI: 1.8–2.3) in 2016 ($P=0.002$).

When looking at post-UMV diversity by age group, both indices confirmed increased diversity among children with 5–14 years of age in the 2016 RV in-season: H′ 1.55 (95% CI: 1.4–1.9) in 2015 versus 2.3 (95% CI: 2.2–2.5) in 2016 ($P=0.003$) and D 0.7 (95% CI: 0.6–0.8) in 2015 versus 0.9 (95% CI: 0.8–0.9) in 2016 ($P=0.02$). Statistically significant change in diversity was not seen among other age groups by in-season (Fig. 1).

Compared with available data on 77 hospitalized children 0–4 years of age from the prevaccine years 2007–2008 published by Soeorg et al,[12] the diversity of genotypes was significantly increased during the postvaccine years 2015–2016 among the age group 0–4: H′ 1.42 (95% CI: 1.2–1.7) during the prevaccine era versus 1.8 (95% CI: 1.7–2) in the postvaccine era ($P=0.008$) and D 0.6 (95% CI:: 0.5–0.7) versus 0.77 (95% CI: 0.75–0.80) ($P=0.011$), respectively.

Clinical Characteristics by Genotype

There was no significant difference by genotype regarding age, gender, time to hospitalization, number of diarrhea episodes, and the need for intravenous rehydration (Table 3).

Genotypes G2P[8], G1P[8], G4P[4], G9P[8] presented with more vomiting episodes compared with others, and genotype G8P[8] was associated with longer hospital stays.

Eighteen cases were detected among vaccinated children (RV1 course completed n=5; RV5 course completed n=10; RV5 course partially completed n=3). Nine cases in 2015 were caused by G1P[8] (n=3), G9P[8] (n=2), G4P[8] (n=1), G12P[8] (n=1), G9P[4] (n=1), and G8P[8] (n=1). In 2016, G9P[8] (n=5) dominated, accompanied by single cases of G9P[4], G12P[8], G2P[6], and G1P[8].

Among vaccinated (n=18) and nonvaccinated (n=339) children with available parameters for VCSS calculation no statistically significant difference in disease severity was seen: mean VCSS 14.1 (SD, 2.4; 95% CI: 12.9–15.3) versus mean VCSS 14.2 (SD, 2.0; 95% CI: 14–14.4), respectively ($P=0.87$).

No significant difference in the mean VCSS by genotype was detected ($P=0.46$) (Table 4).

Compared with prevaccine data available for 69 (90%) patients 0–4 years of age (Hiie Soeorg, personal communication, August 10, 2020), the mean VCSS of children 0–4 years of age increased in 2015–2016: mean VCSS 13 (SD, 2.6; 95% CI: 12.3–13.6) versus VCSS 14.2 (SD, 2.0; 95% CI: 14–14.5), respectively ($P<0.005$).

DISCUSSION

During the second post-UMV RV in-season, we already witnessed significantly decreased circulation of the previously dominant strains G4P[8] and G1P[8] and an increase in genotypes G9P[8], G4P[4], G9P[4], and G2P[8], as well as in genotypes causing less than 1% of cases.

The strength of our study is in capturing 2 RV in-seasons immediately after RV UMV implementation and with good representativeness of 1 country.

Compared with the available data regarding children 0–4 years of age from the prevaccine era,[12] genotype diversity was significantly increased during the postvaccine era among age groups 0–4 years; that is, the children most affected by vaccination. A possible explanation for these findings is a significantly reduced disease burden mimicking off-season periods, when dominance of less common strains has been reported, as was previously shown.[9,22]

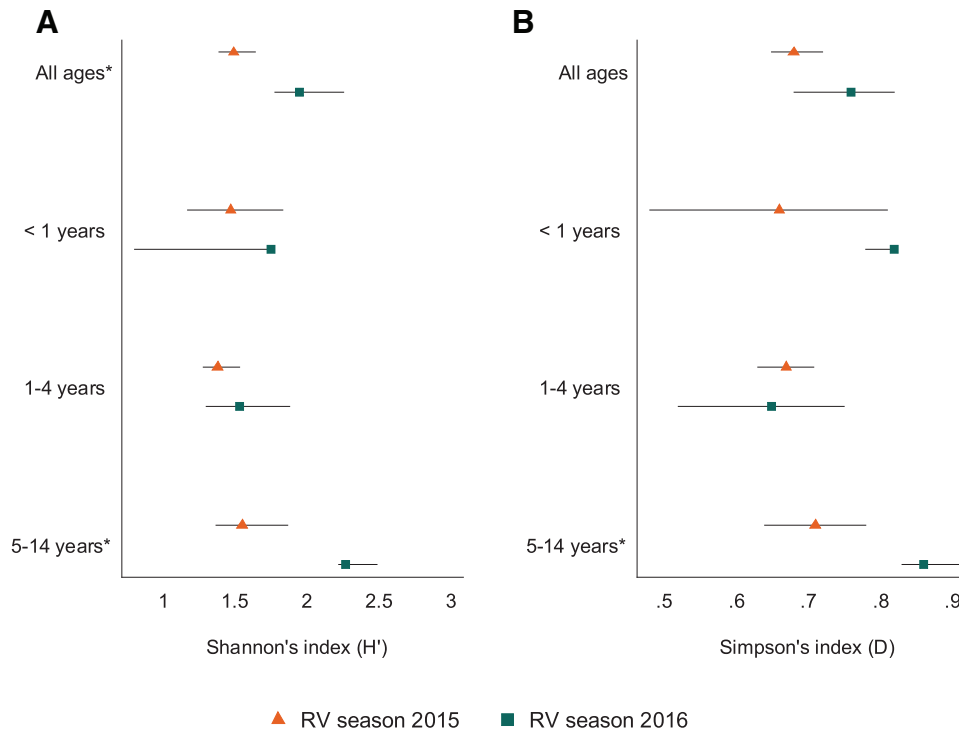

FIGURE 1. RV genotype diversity by age group. (A) Shannon's index with 95% confidence interval, (B) Simpson's index with 95% confidence interval. Age groups with statistically significant changes are marked with an asterisk (*). RV indicates rotavirus. full color online

TABLE 3. Patient Characteristics by Prevailing Genotypes

	Median Age in yrs (IQR)	Gender, Male (%)	Median Time to Hospitalization in d (IQR)	Median Length of Hospital Stay in d (IQR)	Number of Cases With Diarrhea (%)	Median Number of Diarrhea Episodes per 24 h on Admission (IQR)	Number of Cases With Vomiting (%)	Median Number of Vomiting Episodes/d (IQR)	Number of Cases Received Intravenous Rehydration n (%)	Vaccination Status Not Known (%)	Not Vaccinated (%)	Vaccinated (%)
Common genotypes												
G4P[8] (n = 150)	2 (1–4)	84 (56)	1 (0–3)	3 (2–4)	150 (100)	5 (4–8)	139 (93)	6 (4–10)	146 (97)	3 (2)	146 (97)	1 (1)
G1P[8] (n = 101)	2 (1–4)	53 (53)	1 (0–2)	3 (2–3)	101 (100)	4 (3–8)	98 (97)	8 (5–10)	98 (97)	3 (3)	94 (93)	4 (4)
G9P[8] (n = 79)	2 (2–4)	38 (48)	1 (0–3)	2 (2–3)	78 (99)	5 (4–8)	77 (98)	8 (4–10)	79 (100)	2 (3)	70 (89)	7 (9)
G2P[4] (n = 23)	3 (1–4)	12 (52)	1 (0–2)	3 (2–4)	23 (100)	6 (3–12)	23 (100)	6 (3–10)	23 (100)	0 (0)	23 (100)	0 (0)
G12P[8] (n = 10)	3 (1–5)	5 (50)	1 (0–4)	2 (1–3)	10 (100)	4.5 (3–7)	9 (90)	6 (5–10)	10 (100)	0 (0)	8 (80)	2 (20)
Less common genotypes												
G4P[4] (n = 17)	3 (1–4)	5 (29)	1 (0–2)	2 (2–3)	16 (94)	4 (3–7)	17 (100)	8 (6–10)	17 (100)	0 (0)	17 (100)	0 (0)
G2P[8] (n = 14)	3 (1–3)	5 (36)	2 (1–3)	2 (2–3)	14 (100)	4.5 (3–8)	14 (100)	9 (5–12)	14 (100)	0 (0)	14 (100)	0 (0)
G9P[4] (n = 13)	4 (3–6)	7 (54)	1 (0–3)	3 (2–4)	13 (100)	6 (5–10)	13 (100)	5 (3–10)	12 (92)	0 (0)	11 (85)	2 (15)
G8P[8] (n = 5)	2 (0–4)	3 (60)	0 (0–1)	4 (3–4)	5 (100)	5 (4–5)	4 (80)	6.5 (2–10)	5 (100)	0 (0)	4 (80)	1 (20)
G2P[6] (n = 4)	7.5 (3–11.5)	3 (75)	2 (1–12)	2.5 (1.5–6.5)	4 (100)	11 (5.5–15)	2 (50)	5 (5–5)	4 (100)	0 (0)	3 (75)	1 (25)
G9P[9] (n = 3)	2 (1–5)	2 (67)	2 (1–2)	2 (1–5)	3 (100)	7 (3–9)	3 (100)	2 (2–5)	3 (100)	0 (0)	3 (100)	0 (0)

IQR indicates interquartile range.

TABLE 4. VCSS Data by RV Genotype

Genotype	VCSS Available* n	Moderate Disease n (%)	Severe Disease n (%)	Mean VCSS	SD
G12P[8]	10	0 (0)	10 (100)	14.1	2.0
G1P[8]	92	3 (3.3)	89 (96.7)	14.2	2.0
G2P[4]	22	1 (4.6)	21 (95.5)	14.5	2.1
G2P[8]	14	1 (1.14)	13 (92.7)	14.6	1.7
G4P[4]	15	0 (0)	15 (100)	14.4	2.1
G4P[8]	116	5 (4.3)	111 (95.7)	14.2	2.1
G8P[8]	4	1 (25)	3 (75)	12.0	1.4
G9P[4]	12	0 (0)	12 (100)	14.6	1.9
G9P[8]	71	3 (4.2)	68 (95.8)	14.1	2.1
G2P[6]	4	1 (25)	3 (75)	12.8	2.2
G9P[9]	2	0 (0)	2 (100)	12.5	0.7
Total	362	15 (4.14)	347 (95.9)		

VCSS: mild disease <7, moderate disease 7–10, severe disease ≥11 points; maximum score 20 points.

*Cases with all parameters to calculate VCSS documented.

RV indicates rotavirus; VCSS, Vesikari Clinical Severity Scoring System.

In contrast to reports from other countries using RV1 or RV5 for UMV,[6,7,10,11] we did not see dominance of G2P[4] nor G1P[8] post UMV. We believe this may be explained with the proposed hypothesis that both RV vaccines elicit somewhat different protection toward some RV genotypes in different populations.[23] Therefore, the reason for not seeing the dominance of particular strains demonstrated by other countries may possibly be explained by our unique setting: the previously known different genotype pattern in Estonia[12] and successive use of RV5 and RV1 with good vaccination coverage.[16]

We admit it might be premature to suggest that the vaccination influenced genotype profile. The increase of G9P[8] after UMV that we have demonstrated must be interpreted in the context of natural seasonal fluctuations of rotavirus genotypes, as is also suggested by EuroRotaNet surveillance reports. According to EuroRotaNet in 2015/2016, an increase of G9P[8] was seen in several member countries regardless of the presence of RV vaccination programs.[8]

Such a finding emphasizes the fact that the post-UMV genotype profile must always be interpreted along with data available for the general geographic region.

Some studies have raised the question of whether the increasing prevalence of less common genotypes may lead to decreased RV vaccine impact[24]; however, our study shows the good effect of UMV in the context of a heterogenous genotype profile.[22]

Our study also adds to existing knowledge about possible differences in the clinical profile by genotype. Laizane et al[25] has shown that increased vomiting episodes are associated with G1P[8] compared with other genotypes, which is in accordance with the findings in our study. Also, they showed a higher rate of fever and vomiting for genotype G8P[8], which in our study was associated with longer hospital stays. We additionally found genotypes G2P[8], G4P[4], G9P[8] to be associated with more vomiting episodes compared with other genotypes.

As expected, we saw a small set of fully vaccinated children with RVGE. We believe it is most likely associated with the estimated 80–90% effectiveness of RV vaccines against RVGE hospitalizations.[26] However, we cannot totally exclude the possibility that the shedding of RV was a coincidence in these cases, and the real cause of AGE remained undetermined.

There are a few limitations to our study.

We acknowledge that Estonia is a small country, and studies with larger sample sizes over a longer period of time are crucial to further understand the dynamics of RV genotypes post-UMV. The

data presented in our study represent a short pre- and postvaccine period, and any difference could be due to chance or seasonal fluctuations. Without longer pre- and postfollow-up data, we can only state that since the introduction of UMV in Estonia, the circulating RV genotypes are different from those reported in the prevaccine era. Whether emerging variation is due to vaccine pressure or natural fluctuation in RV remains to be determined.

Also, because of financial restrictions, we were not able to analyze stool samples of patients diagnosed with AGE of undetermined etiology at discharge. It is possible that among those patients more RV genotypes could be detected that could lead to a broader understanding of the RV genotype profile post-UMV. However, keeping in mind the possibility to explore the matter in the future, we have preserved all collected stool samples for further studies.

CONCLUSION

Since introduction of UMV in Estonia, the circulating RV genotypes have changed, as compared with those reported in the prevaccine era. Our study adds to knowledge about RV genotype distribution in Europe and provides insight on the clinical profile of prevailing RV genotypes.

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