GENE EXPRESSION
UNIVERSITY

Register here
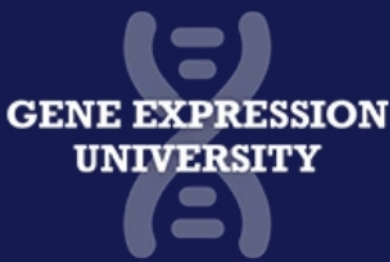
October 06-29

THE ULTIMATE WEBINAR SERIES IN GENE EXPRESSION STUDIES

applied
biosystems
by Thermo Fisher Scientific

WILEY

RESEARCH ARTICLE

Effect of rotavirus vaccine implementation on the prevalence of coinfections with enteric viruses in Norway

Moustafa Gibory MSc[1,2] | Jennifer L. Dembinski PhD[2] | Elmira Flem MD, PhD[3] |
Ildri Haltbakk MSc[2] | Susanne G. Dudman MD, PhD[1]

[1]Department of Microbiology, Faculty of
Medicine, Institute of Clinical Medicine,
University of Oslo, Oslo, Norway

[2]Department of Virology, Norwegian Institute
of Public Health, Oslo, Norway

[3]Department of Infection Epidemiology and
Modeling, Norwegian Institute of Public
Health, Oslo, Norway

Correspondence
Moustafa Gibory, MSc, Department of
Virology, Norwegian Institute of Public Health,
PO Box 4404, N-0403 Oslo, Norway.
Email: moustafa.gibory@studmed.uio.no

Funding information
Norwegian Institute of Public Health

Abstract

Acute gastroenteritis (AGE) is a common illness in both adults and children worldwide and is caused by several microorganisms including viruses, bacteria, and parasites. Rotavirus (RV), which is the main cause of AGE, can occur as a mixed infection with other viruses. The aim of this study is to assess the molecular epidemiology of viral enteric viruses and assess RV coinfections with other enteric viruses and their influence on disease severity before and after RV vaccine introduction in children under 5 years of age. A total of 600 samples collected from children hospitalized for AGE in five large hospitals in Norway, and were analyzed for viral gastroenteritis agents by enzyme immunoassay and quantitative real-time polymerase chain reaction (qRT-PCR). Positive results confirmed either by Sanger sequencing or genotyped by multiplex semi-nested RT-PCR. In total, 243 of the 300 (81%) samples, collected from the prevaccine cohort, were positive for at least one of the four viruses tested in this study. RV was most frequently identified in 82.6% of the samples. In the postvaccine cohort, 114 of the 300 (38%) samples were positive for at least one of the viruses tested. RV found in 36.5% of the samples. Coinfections found less frequently in the postvaccine cohort. Among circulating enteric viruses in Norway, RV is the most important cause of viral gastrointestinal infection. As expected, there were fewer RV positive and fewer coinfections after RV vaccine implementation. The results provide valuable data that can aid in further evaluation of the vaccine impact.

KEYWORDS
AGE, coinfections, norovirus, qRT-PCR, rotavirus, vaccine shedding

1 | INTRODUCTION

Acute gastroenteritis (AGE) in infants and young children is one of the most common public health problems globally which can be caused by several infectious agents such as viruses, bacteria, and parasites.[1] Globally deaths caused by diarrhea have decreased effectively from approximately 2.6 to 1.3 million from 1990 to 2013, and the decline was most observed in children under 5 years of age primarily due to vaccinations.[2,3] At least half of all AGE cases are caused by viruses, especially rotavirus (RV), norovirus (NoV), human adenovirus (HAdV), and human astrovirus (HAsV).[4,5]

RV is the leading cause of diarrhea in infants and young children under 5, and RV type A is the most common species causing a large percentage of AGE worldwide.[6] NoV, which is classified into 10 genogroups (GI-GX),[7] is the second most common viral cause of AGE. GI and GII are assumed to become the most frequent AGE agent in countries that introduced RV vaccines in the universal childhood vaccination program.[8,9]

HAdV is another cause of AGE and is currently considered to be the third leading cause of nonbacterial diarrhea in children.[10] It typically produces a longer period of watery diarrhea and frequent vomiting. The genotypes of HAdV which are associated with AGE are 40 and 41, as well as 38 occasionally.[11,12] HAsV is also a pathogen that can cause outbreaks of acute diarrhea which sometimes require hospitalization, but with much less severe illness than usually associated with RV.[13,14]

There are two oral RV vaccines available worldwide which the World Health Organization recommends in national routine vaccination programs to reduce the burden of RV disease; a monovalent human RV vaccine Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) and a pentavalent bovine-human reassortant vaccine RotaTeq (Merck & Co, Inc, Whitehouse Station, NJ).[15,16] In Norway, a two-dose vaccination program with Rotarix was implemented in 2014 for all children born on or after September 2014. Within the first year of introduction, vaccination coverage reached 94.75% for one dose and 87.70% for two doses.[16,17]

The aim of this study was to map the prevalence of various enteric viruses before and after RV vaccine implementation, focusing on RV coinfections to assess their influence on disease severity in children under 5 in Norway.

2 | MATERIALS AND METHODS

2.1 | Study population

The study population included children under 5 years of age who were hospitalized for AGE in five large Norwegian hospitals (Oslo University Hospital Ullevål, St. Olavs University Hospital in Trondheim, Østfold Hospital Kalnes, Akershus University Hospital, and Stavanger University Hospital in Stavanger). These hospitals represent the Norwegian Enhanced Pediatric Immunization Surveillance network which is a sentinel network for vaccine-preventable diseases. The active sentinel surveillance established at these five hospitals has a catchment population covering about 40% of all Norwegian children less than 5 years of age. Following the introduction of the RV vaccine in Norway, active RV surveillance was initiated in February 2014 before the national rollout of vaccination to monitor the impact of the program. All children less than 5 years of age with AGE who were seeking care in participating hospitals within 10 days of illness onset as well as the children who received at least one dose of the RV vaccine at least 14 days prior to hospital admission were eligible for enrolment. Children with AGE developed 48 hours after hospitalization were excluded from the study.

Children with more than one episode of AGE during the project period were only included once.

A questionnaire was administered to collect clinical and demographic data from all included children such as hospitalization date; unique ID, patient's sex, age, specimen collection date, and type, onset and duration of gastroenteritis symptoms, and length of hospital stay. A Vesikari severity score[18] was used to assess the severity of the gastroenteritis symptoms into severe (score of ≥11), moderate (7-10), or mild (<7). Data were linked to the National Immunization Registry to ascertain RV vaccination status for study participants.

2.2 | Specimen collection

A stool specimen was collected from each enrolled child and tested for enteric viruses at the Norwegian Institute of Public Health. The total number of hospital admissions due to AGE before and after the RV vaccine introduction was 1124 admissions (730 before and 394 after vaccine introduction) with at least one sample. For this study, we randomly selected 600 stool specimens, 300 from the pre- and 300 from the postvaccine cohort. Specimens in the prevaccine cohort were collected between 27 January 2014 and the 4 December 2017, from children between 0.7 and 59 months in age. Postvaccine cohort specimens were collected between 20 November 2014 and the 23 December 2017, and the children were between 0.5 and 37 months in age.

The postvaccine cohort was defined as children born after the 1 September 2014, as these children were offered the RV vaccine through the national childhood vaccination program. The number of specimens needed to achieve a sufficient study power of 0.4 when comparing two groups with a prevalence of 17.5% and 23% with a total of 600 recruited children with AGE was calculated. The prevalence of the gastroenteritis virus agents in the study was estimated based upon our preliminary project data. The largest possible sample size was used in this study but was limited to the number of cases that satisfied the inclusion criteria of the study as described previously.

Specimens were collected either as a bulk stool in a 25 mL container or by using COPAN Regular FecalSwab (470CE; Copan Italy S.p.a, Brescia, Italy) and then frozen at −70°C until further processing.

2.3 | Specimen preparation and viral nucleic acid extraction

For each specimen, a 10% fecal suspension was prepared by adding approximately 100 μL of thin stool (or approximately 50-100 mg of solid stool) to a test tube with 900 μL sample dilution buffer supplied with the RIDASCREEN Kit (R-Biopharm AG, Darmstadt, Germany). Specimens were homogenized using a vortex mixer and subsequently centrifuged at 2000g (13 500 rpm) for 3 minutes before further testing.

Total nucleic acid was extracted using 200 µL of 10% fecal suspension and the Viral NA Small Volume Kit in the MagNA Pure 96 automated nucleic acid extraction instrument according to the manufacturer's protocol (Roche Applied Science, Penzberg, Germany). Samples were eluted into 50 µL and stored at −70°C until further analysis.

2.4 | Enteric virus detection methods

The presence of gastroenteritis virus antigens (RV, HAdV, and HAsV) in 100 µL of the 10% suspension specimens were analyzed by the RIDASCREEN enzyme immunoassay (EIA) (according to the manufacturer's protocol, and the test was carried out in an automated EIA system, DS2 [Dynex Technologies Inc, Chantilly, VA]). Screening for NoV was performed by an in-house quantitative real-time polymerase chain reaction (qRT-PCR)[19,20] using a RotorGene 6000 instrument (QIAGEN, Hilden, Germany).

2.5 | Molecular characterization and confirmation of enteric virus-positive samples

All positive results obtained by EIA and qRT-PCR were confirmed by Sanger sequencing[21,22] with small modifications and genotyping by multiplex semi-nested RT-PCR using previously described protocols.[23,24] The sequencing analysis was carried out using a 3500xL

Genetic Analyzer according to the manufacturer's protocol (Thermo Fisher Scientific Corporation, Waltham, MA).

2.6 | Data analysis

We created four strata for each of the viruses RV, NoV, HAdV, and HAsV and for each strata, contingency tables were created. Fisher's exact test was used to analyze the following combinations of variables: (a) pre-/postvaccine vs sex, (b) pre-/postvaccine vs length of hospital stay, (c) pre-/postvaccine vs Vesikari severity score, and (d) pre-/postvaccine vs age (Table S1). All analyses were performed using the statistical software STATA, SE15 (StataCorp LP, TX).

3 | RESULTS

In the prevaccine cohort, 81% (243/300) were positive for at least one of the four viruses tested in this study with RV being the most frequently identified virus in 82.6% (213/258) of the positive samples, followed by NoV: 8.5% (22/258), HAdV: 7.4% (19/258), and HAsV: 1.5% (4/258), respectively (Figure 1).

On the other hand, in the postvaccine cohort, 38% (114/300) were positive for at least one of the viruses tested. RV was found in 36.5% (42/115) of the positive samples, followed by NoV: 33% (38/115), HAdV: 29.6% (34/115), and HAsV: 0.9% (1/115), respectively (Figure 1).
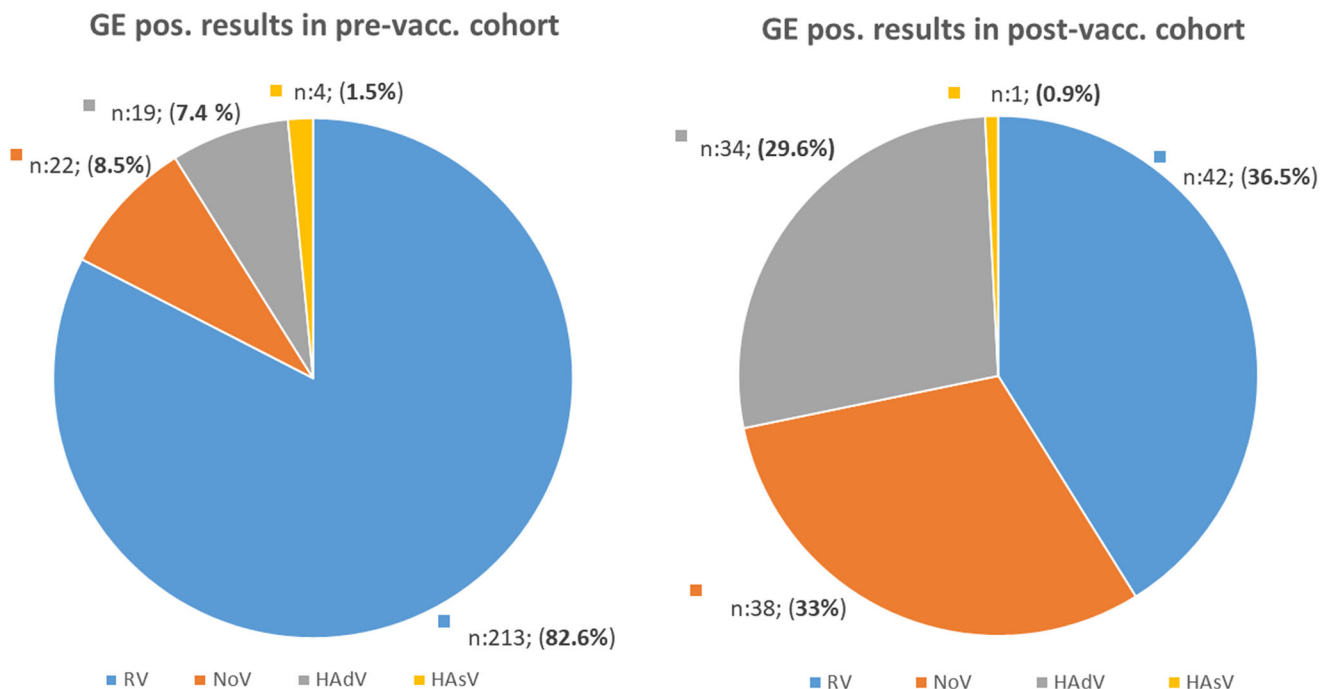
FIGURE 1 Distribution of viral AGE agents for the pre- and postvaccine cohorts. RV was most prevalent in the prevaccine cohort with 82.6% positive, followed by NoV with 8.5%, HAdV with 7.4%, and HAsV with 1.5%. In the postvaccine cohort, the prevalence of RV decreased to 36.5% while NoV increased to 33%, HAdV to 29.6%, and HAsV slightly decreasing to just 0.9%. AGE, acute gastroenteritis; GE, gastroenteritis; HAdV, human adenovirus; HAsV, human astrovirus; NoV, norovirus; RV, rotavirus

In the prevaccine cohort, single virus infections were identified in 74.6% (224/300) of the samples, whereas mixed infections were identified in 6.3% (19/300) of the samples. In the postvaccine cohort, on the other hand, single virus infections were identified in 36% (108/300), and mixed infections were found in just 2.6% (8/300), showing a substantial decrease in the overall prevalence of single and mixed infections after implementation of RV vaccination. Coinfections with RV/NoV were the most common combination and were found in 4% (12/300) of the samples, followed by RV/HAsV in 1.3% (4/300) and RV/HAdV in 1% (3/300).

Coinfections detected in samples obtained from the postvaccine cohort were as follows—RV/NoV: 1% (3/300), RV/HAdV: 1% (3/300), and NoV/HAdV: 0.6% (2/300). A decrease in mixed infections was observed for the combinations RV/NoV and RV/HAsV when comparing to prevaccine samples, although not statistically significant.

Cases with coinfections were separated into four age groups for the pre- and postvaccine cohorts and coinfections were found to occur most frequently in children over 1 year of age (Table 1).

In the postvaccine cohort, 88.6% (266/300) of children received at least one dose of the Rotarix (RV oral vaccine) at least 14 days before hospital admission. Of 226 vaccinated children, 15.8% (42/266) were positive for RV by using both EIA and multiplex seminested RT-PCR methods. We further tested these samples for the presence of RV vaccine strain and found that 9 of 42 samples (21.4%) were positive for the Rotarix vaccine strain. These nine positive samples were excluded from the total wild RV-positive results. Genotypes for the 33 wild RV-positive cases in the vaccinated children were varied and covered 16 different genotypes. The most frequent groups were: G1P[8], G3P[8], and G9P[4].

In the four virus strata, there were no significant associations between pre-/postvaccine cohorts and the variables for sex or length of hospital stay. However, among children with positive RV samples, we found a significant association between pre-/postvaccine cohorts and the Vesikari severity score ($P = .019$). There was a significant reduction in the severity of cases for children with RV-positive samples in the postvaccine cohort, while symptom scores for the children with RV-negative samples in the pre-/postvaccine cohorts remained equal. Additionally, in two strata (children with positive RV or NoV samples), there was a significant association between pre-/postvaccine cohorts and age ($P = .000$ and $P = .001$, respectively). Age could, therefore, be a confounding factor with the Vesikari severity score, thus when estimating the Mantel-Haenszel odds (stratified by age group for children with positive RV or NoV), the P value was increased to .54 and .76, respectively.

4 | DISCUSSION

This is the first study mapping the prevalence of the four most common enteric viruses before and after RV vaccine implementation in Norway with a focus on RV coinfections, to assess the impact on gastroenteritis in children under 5 years of age.

The results show that the prevalence of RV infections decreased substantially, from 82.6% to 36.5% after RV vaccine implementation, while NoV and HAdV increased from 8.5% to 33% and from 7.4% to 29.6%, respectively (Figure 1). This is in line with previous studies, which also found an increase in the frequency of other causes of gastroenteritis after widespread RV vaccination.[25,26] The NoV genotyping results can be found in Table S2.

The prevalence of coinfections in this study is 6.3% (19/300) in the prevaccine cohort vs 2.6% (8/300) in the postvaccine cohort, and the decrease of both the RV prevalence and the RV coinfections after the implementation of the vaccine indicates the potential influence of RV vaccination on AGE in general.

The Vesikari severity score (combined mild and moderate cases) in both the pre- and postvaccine cohort was similar for both single and mixed infections (61% vs 52% and 19.7% vs 20%), respectively, despite the fact that coinfection cases are usually more serious than single infections due to a higher viral load.[27-29]

Shedding of the Rotarix vaccine strain was found in this study in 21.4% (9/42) of the total RV-positive cases. Other studies found that RV vaccine shedding, similarly to other live-attenuated virus vaccines, can occur from the second day after the first dose to several weeks after, and can be transmitted from vaccinated to unvaccinated children. This mainly happens in younger children, primarily at the age when the RV vaccine is given, and corresponds well with the results obtained from this study.[30,31] When conducting routine testing in infants suffering from gastroenteritis, it is important to

TABLE 1 RV coinfections by age in pre- and postvaccine cohorts

Age	Prevaccine cohort				Postvaccine cohort			
	RV/ NoV (%)	RV/ HAdV (%)	RV/ HAsV (%)	NoV/ HAdV (%)	RV/ NoV (%)	RV/ HAdV (%)	RV/ HAsV (%)	NoV/ HAdV (%)
0-5 mo	0	0	0	0	0	0	0	0
6-11 mo	2 (0.6%)	0	0	0	1 (0.3%)	0	0	1 (0.3%)
1-2 y	6 (2%)	0	2 (0.6%)	0	2 (0.6%)	2 (0.6%)	0	1 (0.3%)
>2-4 y	4 (1.3%)	3 (1%)	2 (0.6%)	0	0	1 (0.3%)	0	0
Total	12 (4%)	3 (1%)	4 (1.3%)	0	3 (1%)	3 (1%)	0	2 (0.6%)

Abbreviations: HAdV, human adenovirus; HAsV, human astrovirus; NoV, norovirus; RV, rotavirus.

check their vaccination history to see if RV vaccination was recently given, and consider if testing for the vaccine strain is indicated. Commercial kits used for detecting gastroenteritis agents will not distinguish between RV wild type and vaccine strains, thereby over diagnosing RV infection in infants.

We found no significant association between RV coinfections with sex or length of hospital stay. However, there was a significant association with the Vesikari severity score among RV-positive cases in the postvaccine cohort, and a significant association between pre-/postvaccine and age group distribution among RV- and NoV-positive cases, but no significant association among HAdV and HAsV cases.

There are only a limited number of studies regarding RV coinfections and the effect of RV vaccination on the outcome of gastroenteritis. Different methods or limited numbers of samples were used in these studies, making comparisons difficult,[29,32] but some studies indicate that RV vaccination could reduce the severity of gastroenteritis in the context of coinfections.[33,34]

Our findings highlight the distribution of viral pathogens in the etiology of AGE and their prevalence after RV vaccine implementation in Norway, showing that RV was the most frequent cause of viral AGE followed by NoV despite the extreme decrease in RV cases after vaccine introduction. Furthermore, the results show a relatively high frequency of cases positive with the Rotarix vaccine virus strain which was detected in more than one-fifth of all RV-positive cases.

A possible limitation to this study may be the sample size, which was calculated to have a power of 0.4 when comparing two groups (prevalence 17.5% and 23%) with a total of 600 recruited children with AGE (Table S1). The largest possible sample size was used in this study, but was limited to the number of cases that satisfied the inclusion criteria of the study as described previously. It is therefore possible that the number of coinfections in our study is underestimated.

There is a lack of studies regarding RV coinfections and the possible association with RV vaccination due to a limited observation time after vaccine implementation. Therefore, additional data are needed, as it is important to continue monitoring patterns of viral AGE in Norway to evaluate RV vaccine effectiveness, as well as to assess the vaccine impact in the context of coinfections over a longer period.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

SGD, EF, and MG contributed to the design and implementation of the study. MG, IH, and JLD performed the lab analysis. SGD, EF, JLD, and MG contributed to the data analysis and interpretation. All authors discussed the results and commented on the manuscript. All the authors reviewed and approved the final manuscript to be published.

ETHICS STATEMENT

The Regional Committees for Medical and Health Research Ethics (REK) approved this study. Written informed consent was obtained from parents or legal guardians of all included children.

ORCID

Moustafa Gibory http://orcid.org/0000-0003-3561-8460
Jennifer L. Dembinski http://orcid.org/0000-0001-9259-9572
Elmira Flem https://orcid.org/0000-0002-5099-1960
Susanne G. Dudman http://orcid.org/0000-0001-5047-4982

REFERENCES

1. World Health Organization. *Weekly Epidemiological Record*. 32. Geneva, Switzerland: WHO; 2007:285-296.
2. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet*. 2015;385(9966): 430-440.
3. Lopman BA, Steele D, Kirkwood CD, Parashar UD. The vast and varied global burden of norovirus: prospects for prevention and control. *PLoS Med*. 2016;13(4):e1001999.
4. Chow CM, Leung AK, Hon KL. Acute gastroenteritis: from guidelines to real life. *Clin Exp Gastroenterol*. 2010;3:97-112.
5. Webb A, Starr M. Acute gastroenteritis in children. *Aust Fam Physician*. 2005;34(4):227-231.
6. Braeckman T, Van Herck K, Meyer N, et al. Effectiveness of rotavirus vaccination in prevention of hospital admissions for rotavirus gastroenteritis among young children in Belgium: case-control study. *BMJ*. 2012;345:e4752.
7. Chhabra P, de Graaf M, Parra GI, et al. Updated classification of norovirus genogroups and genotypes. *J Gen Virol*. 2019;100(10): 1393-1406.
8. Koo HL, Ajami N, Atmar RL, DuPont HL. Noroviruses: the leading cause of gastroenteritis worldwide. *Discov Med*. 2010;10(50): 61-70.
9. van Beek J, de Graaf M, Xia M, et al. Comparison of norovirus genogroup I, II and IV seroprevalence among children in the Netherlands, 1963, 1983 and 2006. *J Gen Virol*. 2016;97(9):2255-2264.
10. Liu J, Platts-Mills JA, Juma J, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a re-analysis of the GEMS case-control study. *Lancet*. 2016;388(10051): 1291-1301.
11. Amaral MSC, Estevam GK, Penatti M, et al. The prevalence of norovirus, astrovirus and adenovirus infections among hospitalised children with acute gastroenteritis in Porto Velho, state of Rondônia, western Brazilian Amazon. *Mem Inst Oswaldo Cruz*. 2015;110(2): 215-221.
12. Moyo SJ, Hanevik K, Blomberg B, et al. Prevalence and molecular characterisation of human adenovirus in diarrhoeic children in Tanzania; a case control study. *BMC Infect Dis*. 2014;14:666.
13. Jeong HS, Jeong A, Cheon D-S. Epidemiology of astrovirus infection in children. *Korean J Pediatr*. 2012;55(3):77-82.

14. Lu L, Jia R, Zhong H, et al. Molecular characterization and multiple infections of rotavirus, norovirus, sapovirus, astrovirus and adenovirus in outpatients with sporadic gastroenteritis in Shanghai, China, 2010-2011. *Arch Virol.* 2015;160(5):1229-1238.

15. World Health Organization. Rotavirus vaccines WHO position paper: January 2013–Recommendations. *Vaccine.* 2013;31(52):6170-6171.

16. Hansen Edwards C, de Blasio BF, Salamanca BV, Flem E. Re-evaluation of the cost-effectiveness and effects of childhood rotavirus vaccination in Norway. *PLoS One.* 2017;12(8):e0183306.

17. Bruun TMD, Salamanca BVMP, Bekkevold TMP, et al. Burden of rotavirus disease in Norway: using National Registries for Public Health Research. *Pediatr Infect Dis J.* 2016;35:396-400.

18. Lewis K. Vesikari Clinical Severity Scoring System Manual. Path website. 2011. http://online.fliphtml5.com/xdoe/reem/. Accessed February 28, 2020.

19. Jothikumar N, Lowther JA, Henshilwood K, Lees DN, Hill VR, Vinjé J. Rapid and sensitive detection of noroviruses by using TaqMan-based one-step reverse transcription-PCR assays and application to naturally contaminated shellfish samples. *Appl Environ Microbiol.* 2005; 71(4):1870-1875.

20. Vinje J, Vennema H, Maunula L, et al. International collaborative study to compare reverse transcriptase PCR assays for detection and genotyping of noroviruses. *J Clin Microbiol.* 2003;41(4):1423-1433.

21. Finkbeiner SR, Le BM, Holtz LR, Storch GA, Wang D. Detection of newly described astrovirus MLB1 in stool samples from children. *Emerging Infect Dis.* 2009;15(3):441-444.

22. Sarantis H, Johnson G, Brown M, Petric M, Tellier R. Comprehensive detection and serotyping of human adenoviruses by PCR and sequencing. *J Clin Microbiol.* 2004;42(9):3963-3969.

23. Banerjee I, Ramani S, Primrose B, et al. Modification of rotavirus multiplex RT-PCR for the detection of G12 strains based on characterization of emerging G12 rotavirus strains from South India. *J Med Virol.* 2007;79(9):1413-1421.

24. Iturriza-Gómara M, Kang G, Gray J. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. *J Clin Virol.* 2004; 31(4):259-265.

25. Challappa R, Saito M, Mejia C, et al. Burden of norovirus and rotavirus in children after rotavirus vaccine introduction, Cochabamba, Bolivia. *Am J Trop Med Hyg.* 2016;94(1):212-217.

26. Santos VS, Gurgel RQ, Cavalcante SMM, et al. Acute norovirus gastroenteritis in children in a highly rotavirus-vaccinated population in Northeast Brazil. *J Clin Virol.* 2017;88:33-38.

27. Valentini D, Vittucci AC, Grandin A, et al. Coinfection in acute gastroenteritis predicts a more severe clinical course in children. *Eur J Clin MicrobiolInfect Dis.* 2013;32:909-915.

28. Zhang S-X, Zhou Y-M, Xu W, et al. Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in southwest China. *Infect Dis Poverty.* 2016;5(1):64.

29. de Oliveira Ferreira CE, Raboni SM, Aparecida Pereira L, Nogueira MB, Renaud Vidal LR, Almeida SM. Viral acute gastroenteritis: clinical and epidemiological features of co-infected patients. *Braz J Infect Dis.* 2012;16(3):267-272.

30. Anderson EJ. Rotavirus vaccines: viral shedding and risk of transmission. *Lancet Infect Dis.* 2008;8(10):642-649.

31. Li J-s, Cao B, Gao H-c, et al. Faecal shedding of rotavirus vaccine in Chinese children after vaccination with Lanzhou lamb rotavirus vaccine. *Sci Rep.* 2018;8(1):1001.

32. Tran A, Talmud D, Lejeune B, et al. Prevalence of rotavirus, adenovirus, norovirus, and astrovirus infections and coinfections among hospitalized children in northern France. *J Clin Microbiol.* 2010;48(5): 1943-1946.

33. Pang XL, Koskenniemi E, Joensuu J, Vesikari T. Effect of rhesus rotavirus vaccine on enteric adenovirus-associated diarrhea in children. *J Pediatr Gastroenterol Nutr.* 1999;29(3):366-369.

34. Pang XL, Zeng SQ, Honma S, Nakata S, Vesikari T. Effect of rotavirus vaccine on Sapporo virus gastroenteritis in Finnish infants. *Pediatr Infect Dis J.* 2001;20(3):295-300.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.