Identification of Strains of RotaTeq Rotavirus Vaccine in Infants With Gastroenteritis Following Routine Vaccination

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Background. RotaTeq vaccine was introduced into the Australian National Immunisation Program in 2007. This study identified and characterised rotavirus strains excreted by infants who presented with symptoms of gastroenteritis following recent RotaTeq vaccination.

Methods. Fecal samples (N = 61) from children who developed gastroenteritis following recent RotaTeq vaccination were forwarded to the Australian Rotavirus Surveillance Program (ARSP). RotaTeq-positive samples were genotyped and regions of the *VP3*, *VP4*, *VP6*, and *VP7* genes were sequenced. Also, 460 rotavirus-positive ARSP routine surveillance samples were analyzed by dot-blot Northern hybridization to detect RotaTeq vaccine–derived strains circulating in the community.

Results. Thirteen of the 61 samples collected from infants developing gastroenteritis after RotaTeq vaccination contained vaccine-derived (vd) rotavirus strains. Of these, 4 contained a vdG1P[8] strain derived by reassortment between the G1P[5] and G6P[8] parental vaccine strains. Northern hybridization analysis of 460 surveillance samples identified 3 samples that contained RotaTeq vaccine–derived strains, including 2 vdG1P[8] reassortant vaccine strains.

Conclusions. During replication and excretion of RotaTeq vaccine, reassortment of parental strains can occur. Shedding of RotaTeq vaccine strains in 7 of 13 infants was associated with underlying medical conditions that may have altered their immune function. The benefits of vaccination outweigh any small risk of vaccine-associated gastroenteritis.

Rotavirus is the principal etiological agent of severe acute gastroenteritis in young children worldwide. Rotavirus causes 114 million episodes of diarrhea annually worldwide, resulting in 24 million clinic visits, 2.4 million hospitalizations, and an estimated 527 000 deaths in children <5 years of age [1, 2].

Rotavirus belongs to the Reoviridae virus family and has an 11-segment double-stranded RNA (dsRNA) genome that encodes 6 structural viral proteins (VP1-4, VP6, and VP7) and 6 nonstructural proteins (NSP1–6) [3]. The 2 outer capsid proteins, VP7 and VP4, elicit genotype-specific neutralizing antibody responses and classify group A rotavirus strains into G (glycoprotein) and P (protease-sensitive) genotypes, respectively [3]. Genotypes G1P[8], G2P[4], G3P[8], G4P[8], and G9P [8] cause the majority of rotavirus disease worldwide [4]. Rotavirus is able to evolve rapidly via genetic drift, genomic rearrangement, duplications, and deletions of gene sequence and zoonotic transmission of strains.

The segmented genome also facilitates genetic shift (reassortment) between 2 different virus strains coinfecting a cell [5].

Children experience several rotavirus infections early in life. Primary infection generally results in the most severe disease, with subsequent infections being milder or asymptomatic. Primary infection typically induces homotypic protection (against infecting genotype) with secondary infections broadening immune responses to provide heterotypic protection (against different genotypes). Vaccines aim to mimic the protection afforded by natural rotavirus infection [6].

Two rotavirus vaccines, Rotarix (GlaxoSmithKline) and RotaTeq (Merck), are licensed in numerous countries worldwide and are included in several national vaccination programs [7–9].

Rotarix is a live-attenuated human monovalent strain, G1P [8]. The vaccine is administered in a 2-oral-dose schedule, typically at 2 and 4 months of age [10]. RotaTeq is a live-attenuated pentavalent vaccine. The 5 genetically distinct human-bovine reassortant rotavirus strains each contain a human gene, encoding 1 of the outer capsid proteins, within a bovine WC3 strain backbone (G6P[5]). Four of the reassortant strains—WI79-9, SC2-9, WI78-8, and BrB-9—have a *VP7* gene encoding genotypes G1, G2, G3, and G4, respectively, and 1 reassortant strain WI79-4 that contains the *VP4* gene encoding P[8]. While the RotaTeq vaccine strains possess a bovine WC3 backbone, the W179-9 and SC2-9 strains possess *VP3* genes of human rotavirus strain origin. RotaTeq is administered as a 3-dose schedule, recommended at 2, 4, and 6 months of age [11].

The majority of immunocompetent children with severe primary wild-type gastroenteritis routinely shed rotavirus for 7–10 days. However, one-third often continue to shed virus for up to 21 days, with examples of shedding up to 51 days documented [12]. Rotarix and RotaTeq vaccine strains can replicate in the human small intestine. Shedding of the Rotarix vaccine strain was detected during clinical trials in 35%–80% of healthy vaccine recipients 7 days after the first dose and 11%–29% after the second dose [7]. In phase 3 studies of the RotaTeq vaccine, 8.9% of vaccine recipients shed the vaccine following the first dose, 0% after the second dose, and 0.3% after the third dose. Shedding, as detected by plaque assay (detects viable, intact virus), was observed as early as day 1. Shedding peaked between days 4 and 6. The quantity of RotaTeq vaccine virus shed was relatively low, approximately 0.001%–1% of the administered dose [13]. A postlicensure study in a primary care practice demonstrated that rotavirus antigen was detected by enzyme immunoassay (detecting viable and degraded virus) in 21.4% of subjects after the first dose, with vaccine shedding highest at 6–8 days after vaccine administration. The shed virus was viable and could be cultivated in tissue culture [14].

The rationale for rotavirus vaccination in Australia primarily arises from the high morbidity associated with infection; the mortality rate is extremely low. Prior to vaccine introduction, 115 000 general practitioner consults, 22 000 emergency department presentations, and 10 000 hospitalizations in children <5 years of age could be attributed to rotavirus infection annually, resulting in direct medical costs of AUD$30 million [15]. Rotarix and RotaTeq were introduced into the Australian National Immunisation Program in July 2007. Each state and territory independently evaluated which vaccine to implement. Victoria, Queensland, and South Australia selected RotaTeq, and the remaining states and territories selected Rotarix, with Western Australia changing to RotaTeq in 2009 [16]. Overall, vaccine coverage reached 87% for 1 dose and 84% for full vaccine course by December 2008 [17].

The primary aim of this study was to identify and characterize rotavirus isolates from infants who had recently been administered RotaTeq vaccine and who had subsequently presented at tertiary care facilities with any symptom of gastroenteritis. The secondary aim of this study was to identify any RotaTeq vaccine strains circulating in communities using the vaccine.

METHODS

Sample Collection

Fecal samples (n = 33) were collected from infants who presented to the Royal Children's Hospital, Victoria, with any symptoms of gastroenteritis within 2 weeks of being immunized with RotaTeq. All had been followed up in the state-based specialist vaccine safety service (Surveillance of Adverse Events Following Vaccination In the Community, SAEFVIC; www.saefvic.org.au) between 1 July 2007 and 1 September 2010. Fecal samples were collected from infants in Queensland (n = 27) and Western Australia (n = 1) whose physicians noted gastroenteritis after recent RotaTeq vaccine administration. In each state RotaTeq is the vaccine included in the childhood immunization program. Prior to inclusion in the study, each sample was tested for the presence of enteric pathogens by the microbiology and virology pathology services at the site of collection. Samples were routinely tested for the presence of ova, cysts, parasites, *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, *Plesiomonas*, *Yersinia*, adenoviruses, and *Norovirus*. None of the pathogens listed were detected in any samples included in the study. Review of the medical records was conducted on 6 patients admitted to the Royal Children's Hospital, Victoria, and 2 patients showed concomitant infection with parainfluenza virus 3 or cytomegalovirus.

Rotavirus-positive fecal samples (N = 460) collected from children presenting with severe gastroenteritis to the emergency departments of Royal Children's Hospital, Victoria; Royal Children's Hospital, Queensland; and Queen Elizabeth Medical Centre, Western Australia, that had been genotyped as part of the Australian Rotavirus Surveillance Program

(ARSP) between 2008 and 2010 were also analyzed. These samples were tested for the presence of enteric pathogens as described above.

Extraction of Viral RNA

Rotavirus dsRNA was extracted from 20% fecal suspensions using an RNA extraction kit (QIAamp Viral RNA mini kit [spin protocol], Qiagen) in accordance with the manufacturer's instructions.

Amplification of RotaTeq-Specific Genes

To determine the presence of a vaccine or wild-type rotavirus strain, a portion of the inner capsid gene, VP6, was amplified using the Superscript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen), and the bovine primers BovVP6F and BovVP6R and the human primers Rot-3 and Rot-5 at a concentration of 10 μM were used [18]. Five microliters of RNA was denatured at 97°C for 3 minutes and quenched on ice. The denatured dsRNA was reverse-transcribed at 45°C for 30 minutes, with polymerase chain reaction (PCR) activation at 95°C for 15 minutes, followed by 40 cycles of amplification: 94°C for 30 seconds, 45°C for 45 seconds, and 75°C for 75 seconds, with a final extension at 70°C for 7 minutes in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems).

Portions of gene segments encoding proteins VP3, VP4, and VP7 were reverse-transcribed and amplified by Reverse transcription PCR (RT-PCR) as described above, with the modification of an elongation time of 5 minutes. The standard primer sets Beg9/End9 or VP7F/VP7R were used to amplify VP7. VP3 was amplified with the primer sets RTeq2.20F and RTeq3.1086R and VP4 was amplified using RTeq4.start and con2 [19–21]. The RTeq primers were designed based on the published sequence of the G6P[8] parental vaccine strain (Supplementary Table 1) GenBank accession numbers GU565043 and GU565044.

Genotyping PCR

RotaTeq-positive samples were subjected to a 2-step heminested multiplex RT-PCR to determine G and P genotype. The One-Step RT-PCR Kit with Omniscript, Sensiscript, and HotStartTaq DNA polymerase (Qiagen) was utilized with genotyping primers and protocol previously described [19–23]. Bovine P genotyping was conducted on all samples using the previously established protocol [24].

Sequence Analysis

PCR amplicons were purified via gel extraction and spin column purification using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's protocol. Purified DNA, together with oligonucleotide primers, was sent to the Australian Genome Research Facility, Melbourne, for sequence analysis using an Applied Biosystems 3730 × l DNA Analyzer.

Sequence data were analyzed using the Sequencher software program version 4.1 (Gene Codes Corp). Sequence identity was determined using the BLAST server on the GenBank database at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), and sequence alignments were constructed using MEGA software, version 4 [25].

Dot-Blot Northern Hybridization

A probe mix was generated by pooling individual RotaTeq-specific probes directed to 7 gene segments, encoding VP1, VP2, VP6, NSP1, NSP2, NSP3, and NSP4 that were generated using PCR digoxigenin labeling mix (Roche). RT-PCR amplification was conducted using the same conditions as described previously. The primers used are listed in Supplementary Table 1.

Denatured rotavirus dsRNA was spotted onto nylon membranes (Roche) and cross-linked via UV exposure. Prehybridization washes were performed at 52°C, prior to hybridization at 52°C for 16 hours. Posthybridization washes were performed at room temperature and at 68°C. The bound probes were detected using antidigoxigenin antibody conjugated to alkaline phosphatase (Roche) and visualized using the chemiluminescent substrate CDP-Star (Roche) and exposure to radiographic film (Amersham Hyperfilm MP).

RESULTS

Sixty-one fecal specimens were collected between 1 July 2007 and 1 September 2010 from infants with temporally associated acute gastroenteritis after RotaTeq vaccination. No sample tested positive for the presence of other enteric pathogens including parasites, *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, *Plesiomonas*, *Yersinia*, adenoviruses, and *Norovirus*. To determine the presence of rotavirus, VP6 RT-PCR amplicons were sequenced and compared with the known sequence of the RotaTeq vaccine and wild-type strains. Forty-three samples were confirmed as rotavirus positive, and 30 were identified as containing wild-type rotavirus strains. Of the 13 samples identified as containing a strain from the RotaTeq vaccine, 2 samples contained a single vaccine strain (G1P[5]); 5 samples contained 2 vaccine strains, as shown by the simultaneous presence of P[8] and P[5] P genotypes; and 2 samples contained at least 4 of the vaccine strains. The remaining 4 samples contained single vaccine-derived (vd) G1P[8] (denoted vdG1P[8] hereafter) strains that were analyzed further (Table 1). The remaining 18 samples tested negative for the presence of rotavirus. These samples could represent cases of diarrhea due to other causes.

To identify whether the vdG1P[8] strains were reassortants derived from the coinfection of 2 parental vaccine strains or from coinfection between a vaccine and wild-type strain, sequence analysis of genes encoding VP3, VP4, VP6, and VP7

Table 1. Summary of Infants With RotaTeq Vaccine Strains Identified in Association With Gastroenteritis

Sample	Age, mo	VP7 Genotype	VP4 Genotype	Time Between Vaccination and Sample Collection	Underlying Medical Condition
Patient 1	2.5	G1	P[8]/P[5]	4 d	Present
Patient 2[a,b]	9	G1	P[8]	3 mo	Present
Patient 3	4	G1	P[8]/P[5]	12 d	Not reported
Patient 4	2	G1/G2/G3/G4	P[8]/P[5]	4 d	Present
Patient 5	3	G1	P[8]/P[5]	6 d	Not reported
Patient 6[a]	5	G1	P[8]	7 d	Present
Patient 7[a]	2	G1	P[8]	6 d	Present
Patient 8	2	G4	P[8]/P[5]	2 d	Present
Patient 9[a]	2	G1	P[8]	Unknown	Present
Patient 10	2	G1	P[5]	7 d	Not reported
Patient 11	Unknown	G1	P[5]	Unknown	Not reported
Patient 12	4	G1	P[8]/P[5]	Unknown	Not reported
Patient 13	2	G1/G3/G4	P[8]/P[5]	Unknown	Not reported

[a] Denotes patients identified as shedding a single vaccine-derived G1P[8] reassortant strain (between the G1P[5] and G6P[8] strains).
[b] Patient 2 had severe combined immune deficiency and shed RotaTeq vaccine strain virus for 7.5 months.

was performed. All samples exhibited a high degree of nucleotide homology (99.5%–100%) with the published RotaTeq sequences (Table 2). Analysis of the *VP3* gene sequence allowed determination of whether the G1P[5] or G6P[8] parental strain provided the virus backbone. All reassortant strains showed a high degree of nucleotide homology (99.6%–100%) to the G6P[8] strain. Therefore, it was concluded that the vdG1P[8] strains detected in samples from patients 2, 6, 7, and 9 were reassortants between the vaccine reassortant strain G1P[5] and the vaccine reassortant strain G6P[8].

To explore whether RotaTeq vaccine strains were circulating and causing disease within the infant population, 460 fecal samples were collected between 2008 and 2010 as part of routine ARSP rotavirus surveillance. Specimens were collected from states using RotaTeq (Victoria, Queensland, and Western Australia) and were screened by dot-blot Northern hybridization. Sequence analysis of the *VP3*, *VP4*, *VP6*, and *VP7* genes of 3 positive samples revealed that 1 sample contained the G6P[8] vaccine strain and 2 samples contained vdG1P[8] reassortant strains, exhibiting 99.7%–100% nucleotide homology to the published sequence of RotaTeq (Table 3, patients 14 and 15). Both samples exhibited 100% homology to the *VP3* gene of the G6P[8] reference strain (Table 2).

DISCUSSION

The introduction of rotavirus vaccines into the Australian childhood immunization program has resulted in a significant reduction in rotavirus-associated hospitalizations, emergency room visits, and episodes of gastroenteritis [16]. The rotavirus

Table 2. Percentage Nucleotide Homology of Reassortant vdG1P[8] Vaccine Strains to the Published Sequence of the RotaTeq Vaccine Component Strains

Patient	VP6 Gene	VP4 Gene	VP7 Gene	VP3 Gene–Nucleotide Homology to Specific Strains	
				G6P[8]	G1P[5]
Patient 2[a]	100% (G6P[8])	99.5% (P[8]) {3}	99.2% (G1) {8}	99.6% (P[8]) {4}	76.5% (G1) {239}
Patient 6[a]	100% (G6P[8])	100% (P[8])	99.8% (G1) {2}	100% (P[8])	76.9% (G1) {241}
Patient 7[a]	100% (G6P[8])	100% (P[8])	99.9% (G1) {1}	100% (P[8])	76.9% (G1) {241}
Patient 9[a]	100% (G6P[8])	100% (P[8])	100% (G1)	100% (P[8])	76.9% (G1) {241}
Patient 14[b]	100% (G6P[8])	100% (P[8])	100% (G1)	100% (P[8])	76.9% (G1) {241}
Patient 15[b]	99.7% (G6P[8]) {1}	99.8% (P[8]) {2}	99.9% (G1) {1}	100% (P[8])	76.9% (G1) {241}

Abbreviation: {}, number of nucleotide changes.
[a] Denotes reassortant strains isolated from patients with RotaTeq vaccine–associated gastroenteritis.
[b] Denotes reassortant strains isolated from patients characterized by Northern hybridization.

Table 3. Summary of Samples Identified Using Northern Hybridization as Containing RotaTeq Vaccine

Sample	Age, mo	VP7 Genotype	VP4 Genotype	Time Between Vaccination and Sample Collection
Patient 14[a]	2	G1	P[8]	Unknown
Patient 15[a]	Unknown	G1	P[8]	Unknown
Patient 16	2	G6	P[8]	Unknown

[a] Denotes patients identified as shedding a single vaccine-derived G1P[8] reassortant strain (between the G1P[5] and G6P[8] strains).

vaccine coverage rate varies between states and in 2008 a complete course (2–3 doses) was achieved in 81.3% of infants in Victoria, 80.7% in Queensland, and 78.7% in Western Australia [26]. In 2 Australian states using RotaTeq vaccine, the introduction has been associated with significant decreases in hospitalization of 53%–68% (2007–2009) in Victoria, and a reduction in rotavirus notification by 53% and 65% in 2007 and 2008, respectively, which was associated with significant vaccine effectiveness in Queensland [16, 27, 28]. This decrease in rotavirus detection and hospitalizations after vaccine introduction has been documented in many settings including the United States and Nicaragua [29, 30]. Data from the three phase 3 clinical trials investigated the tolerability of RotaTeq found that although the incidence of fever and irritability did not differ between vaccine and placebo recipients in the week following the first dose, diarrhea and vomiting occurred more frequently among vaccine recipients than among placebo recipients (10.4% vs 9.1% and 6.7% vs 5.4%, respectively) [13].

In this postlicensure study, we describe the identification of RotaTeq vaccine–derived strains temporally associated with postvaccine diarrhea in infants admitted to 3 Australian pediatric hospitals. RotaTeq vaccine strains were identified in 21.3% (13 of 61) of infants who developed diarrhea within 2 weeks of vaccine administration. On the basis of patient age at the time of sample collection, the dose of vaccine resulting in symptomatic excretion could be determined. Seven infants developed symptomatic excretion after the first dose of RotaTeq and 4 patients developed symptomatic excretion after the second dose. The age of a single patient was unknown, so vaccine dose could not be determined. One patient had severe combined immune disorder (SCID) and received the scheduled 3 vaccine doses and experienced mild gastroenteritis after the first dose, which worsened with subsequent doses. It is likely that strains originating from the third dose were characterized in this study. Although the length of time of RotaTeq vaccine excretion is unknown for all but 1 of these patients, the length of time between vaccine administration and the infant presenting to tertiary care facilities with symptoms of gastroenteritis was known for 9 patients. With the exception of the SCID patient who presented 3 months after the final RotaTeq dose, the infants in this study presented 2–12 days after vaccination. Postlicensure studies have detected shedding from day 2 to day 9 after vaccination with primary RotaTeq dose [14]. In a postlicensure study of excretion of RotaTeq in premature infants following the primary dose, excretion was detected from day 1 to day 15 [31].

Of the 13 RotaTeq-positive samples identified in infants, 4 excreted a vdG1P[8] vaccine-derived virus produced by reassortment between 2 of the vaccine strains, G1P[5] and G6P[8]. Sequence analysis of the VP7 and VP4 genes confirmed that the outer capsid genes were vaccine derived, and the RotaTeq WC3 bovine backbone was confirmed by sequence analysis of the VP3 and VP6 genes. The presence of the bovine VP3 also suggests that the VP7 gene was obtained from the G1P[5] strain and was reassorted into the G6P[8] strain, since only the G1P[5] and G2P[5] strains contain a human VP3 gene [32].

Two previous reports have detailed reassortment events between RotaTeq vaccine strains. A single RotaTeq vaccine–derived reassortant G1P[8] was identified in association with horizontal transmission from a 2-month-old vaccinated infant to a 30-month-old sibling, who developed a symptomatic infection in the absence of other enteric pathogens. Neither child was immunocompromised upon initial or follow-up evaluations [33]. During a phase 2 trial of a quadrivalent precursor vaccine to RotaTeq, 2 vaccine-derived reassortant G1P[8] strains were identified (after the first dose), among 161 vaccine recipients, 7 of whom excreted vaccine virus. In this instance, the reassortant strains caused asymptomatic infection [7]. It is possible that a reassortant RotaTeq vdG1P[8] strain may possess characteristics of increased virulence compared with the original "naturally attenuated" bovine strain [33]. For example, the combination of the 2 human outer capsid proteins could enhance cell binding and entry into human intestinal cells.

In nature, rotavirus is able to evolve rapidly in response to immunological pressure. There is evidence that gene reassortment is a common event. Full genome sequence analysis of G3P[8] and G4P[8] human strains from Washington, D.C., has revealed intragenotypic diversity in human strains, presumably by reassortment events. Human–animal reassortants have often been identified as unusual human strains [34, 35]. Full genome analysis of animal strains (bovine, porcine, and simian) has revealed that they possess divergent gene constellations that possibly originated from a combination of interspecies transmission and gene reassortment [36].

This study suggests that infants who received RotaTeq may have vaccine virus strains in their stool shortly after vaccination. It should also be noted that similar reassortment is possible between the human rotavirus–derived vaccine Rotarix and wild-type strains. However, identification of such reassortants requires much detailed sequence analysis to confirm vaccine derived genes. Among the 460 rotavirus samples collected by

the ARSP from children with gastroenteritis admitted to hospitals in states using RotaTeq, 3 samples tested positive for RotaTeq vaccine strains. The vaccination status of these individuals is unknown; however, 2 of the patients were 2 months of age and thus likely to represent cases where infants had been recently vaccinated, and community circulation of vaccine strains cannot be confirmed.

Rotavirus vaccination can provide indirect and direct protection to the unvaccinated community. Postlicensure studies have demonstrated a decrease in rotavirus disease in children ineligible for vaccination owing to age, indicating a reduction in viral transmission in the community [37]. The shedding of vaccine strains may provide direct protection to unvaccinated individuals. The transmission of rotavirus vaccine strains to unvaccinated children could increase herd immunity to rotavirus disease and could be particularly beneficial in populations with low vaccine coverage. A postlicensure study in the Dominican Republic found that transmission of Rotarix occurred in 18.8% of twin pairs (15/80); 4 patients showed signs of having seroconverted the transmitted vaccine strain based on anti-rotavirus immunoglobulin A antibody levels [38]. However, the prolonged shedding of vaccine strains raises potential concerns. The transmission of vaccine strain virus to contacts, including those who are immunocompromised, could result in symptomatic infection. Seven cases of horizontal transmission of rotavirus vaccine strains causing symptomatic infection have been reported by the United States passive Vaccine Adverse Event Reporting System, including transmission to adult relatives and 1 case of transmission to an immunocompromised father.

This study raises the possibility that RotaTeq vaccine strains might infect an individual with an altered immune system more readily than previously understood. For example, 1 of the vdG1P[8] reassortant strains was identified in a patient with SCID. Prolonged excretion in this individual was resolved following successful cord-blood transplantation [39]. Diarrheal disease in severely immunocompromised individuals after rotavirus vaccine administration is described in the medical literature, with at least 9 cases documented, and vaccination with both RotaTeq and Rotarix has been contraindicated in infants diagnosed with SCID since December 2009 and February 2010, respectively [40, 41]. In this study, underlying medical conditions were documented in 7 patients with RotaTeq vaccine–associated diarrhea, including an infant with Down syndrome and another with a history of necrotizing enterocolitis. These conditions are not contraindications to rotavirus immunization. However, there is a need for further research into conditions that may lead to an increased susceptibility to rotavirus disease even after immunization.

One of the limitations of this study was case ascertainment. The SAEFVIC service is a passive surveillance system, relying predominantly on healthcare professionals to report adverse events following immunization. In addition, not all cases temporally reported to have gastroenteritis symptoms after RotaTeq vaccination had a fecal sample collected, leading to case ascertainment bias. There was also limited clinical information on a number of cases, including the history of underlying medical conditions. The timing of the symptoms after rotavirus immunization and the severity of diarrhea and associated dehydration level were also not routinely captured. To further investigate the findings in this study, active data linking is needed between emergency hospital presentations with gastroenteritis symptoms occurring within 2 weeks of rotavirus immunization.

CONCLUSION

The benefits of the rotavirus vaccine program in Australia and internationally have been clearly established in preventing severe disease and death from rotavirus disease [16, 27–30]. Given the right set of circumstances, vaccine strains can cause disease in individuals with underlying medical conditions associated with immunosuppression. This study has documented a total of 16 cases of vaccine strains in children with diarrhea, with 6 of the cases involving reassortment events between 2 of the reassortant vaccine strains. While our study does confirm that reassortment between RotaTeq vaccine strains can occur in postlicensure settings, it should be highlighted that these cases represent a very small proportion of all the individuals vaccinated. The risk posed by wild-type infection far outweighs the risks associated with the rare event of vaccine-associated disease.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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