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Rotavirus shedding in premature infants following first immunization☆

Candice K. Smith[a,*], Monica M. McNeal[b], Nicole R. Meyer[b], Simone Haase[b], Cornelia L. Dekker[a]

[a] Stanford University School of Medicine, 300 Pasteur Drive, G312, Stanford, CA 94305, USA
[b] Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, ML6014, Cincinnati, OH 45229, USA

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ABSTRACT

Objective: There is limited data regarding rotavirus vaccine shedding in premature infants. We describe the natural history of rotavirus shedding in premature infants in the 2-week period following first immunization with RotaTeq®, the pentavalent rotavirus vaccine (RV5), and the risk for symptomatic transmission to household contacts (HHC).
Patients and methods: A prospective pilot study of 15 premature infants of gestational ages 26–34 weeks immunized with RV5 between 6 and 14 weeks chronological age on discharge from the NICU was conducted. Stool samples collected in the following 2 weeks and analyzed for rotavirus antigen by enzyme immunoassay (EIA), cell culture, and RT-PCR. Solicited adverse events were collected on study subjects and any symptoms of fever, vomiting and diarrhea in HHC.
Results: Rotavirus antigen shedding after immunization was detected, with positive rotavirus EIA results in 53.3% of premature infants and in 22.1% of 86 stool samples collected. Shedding rates by RT-PCR were higher with 86.7% of infants and 76.7% of samples being positive. Only 42% of EIA positive samples were positive by cell culture (8/86 total samples, 9.3%). None of 53 HHC reported symptoms of rotavirus infection during the 4 weeks following immunization of the infants.
Conclusions: The findings of this study demonstrate that premature infants have positive stools by EIA, viral culture, and RT-PCR at varying time points during 2 weeks following first-dose immunization with RV5. RT-PCR shedding rates need to be clinically evaluated in the context of virus quantification by cell culture, which was low. No symptomatic transmission to HHC was detected in this study, supporting low transmissibility of vaccine virus shed by these infants born prematurely.

1. Introduction

Premature infants who become infected with rotavirus are at increased risk for hospitalizations and have a clinically different spectrum of disease including gastroenteritis, bloody or mucoid stools, intestinal dilation, and necrotizing enterocolitis [1,2]. Rotavirus vaccination of infants has decreased the burden of disease [3,4]. RotaTeq® is a live, oral, pentavalent rotavirus vaccine, referred to as RV5, and was licensed in 2006.

As part of the large-scale, blinded, placebo-controlled RV5 efficacy and safety trial (REST), more than 2000 premature infants from 25 to 36 weeks gestational age (median age of 34 weeks) were evaluated; a 100% rate reduction in hospitalizations and emergency department visits and a 73% rate reduction in rotavirus gastroenteritis of any severity were demonstrated up to 2 years following three doses with RV5 vaccine [5]. The frequency of serious adverse events in premature infants was comparable in the vaccine (5.5%) versus the placebo groups (5.8%).

The REST study assessed shedding of rotavirus by viral culture with use of a plaque assay and RNA electropherotyping from stool specimens that were shown to be rotavirus positive by a sensitive enzyme-linked immunosorbent assay (EIA) in a subset of infants at a single time point, on day 4–6 after each dose. Authors found 17/134 (12.7%) infants positive after the first dose, 0/109 positive after the second dose, and 0/99 positive after the third dose [6]. The Merck package insert summarizes shedding data in a larger cohort of 360 term healthy infants and describes an 8.9% (32/360) shedding rate by EIA on day 4–6 after the first dose of RV5, 0% (0/249) after the second dose, and 0.3% (1/385) after the third dose [7]. A recent study looked at rotavirus shedding on days 1–9 post-first-immunization by EIA in term infants and found a rate of 21.4% (22/103) [8]. The REST study provides very limited data on premature infant shedding of rotavirus, as it only assessed 7 premature infants, none of whom had shedding detected on day 4–6 after any dose of vaccine [5]. Based on these data, the ACIP and AAP recommended that pre-

Abbreviations: RV5, pentavalent rotavirus vaccine; HHC, household contact; EIA, enzyme immunoassay.

☆ The findings and conclusions in this article are those of the authors and do not necessarily represent the official position or views of the Centers for Disease Control and Prevention.

* Corresponding author. Permanent address: 770 Welch Road, Stanford, CA 94305, USA. Tel.: +1 801 580 9967; fax: +1 451 981 1065.
E-mail address: candice.kendell@gmail.com (C.K. Smith).

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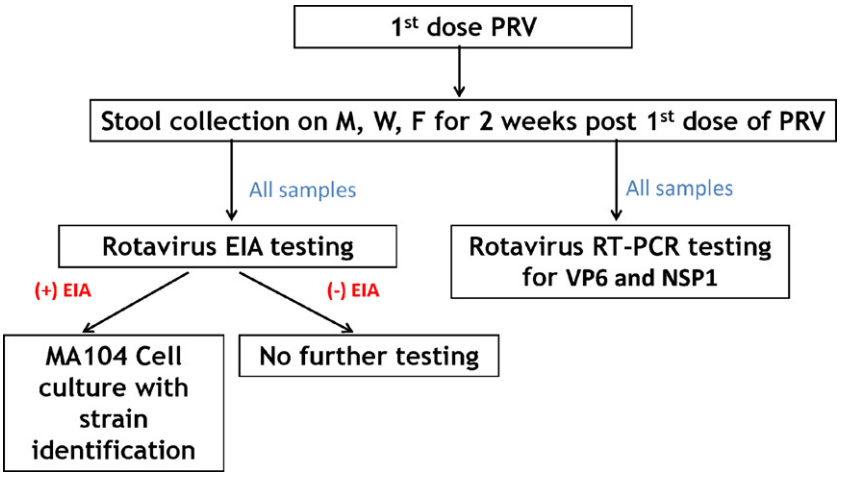

Fig. 1. Flow chart describing methods for sample collection and laboratory analysis.

mature infants should receive rotavirus vaccination on discharge from the hospital nursery or after discharge on the same schedule as term infants if they are between 6 and 14 weeks of age and clinically stable [5,9].

Many Neonatal Intensive Care Units do not vaccinate their patients with rotavirus vaccine because of concerns about the risk of virus shedding that could theoretically take place within the hospital setting. As a result, some premature infants with prolonged hospitalization miss the strict age window for administering the first dose of vaccine and therefore remain unvaccinated and at an increased risk for severe rotavirus infection [1].

The concern about potential infectivity of recently immunized inpatient premature infants is theoretically plausible based on the ease of wild type rotavirus transmission in daycare centers and of the low infectious dose required for adults [10–12]. Recently, a case of sibling-to-sibling transmission of vaccine-derived rotavirus gastroenteritis has been described [13]. We conducted this pilot study of the rate of rotavirus shedding in stools of premature infants following a first dose of RV5, to more thoroughly study virus shedding in this population, and to provide data regarding transmission to their contacts at home.

2. Patients and methods

2.1. Study population

The study was a pilot prospective trial conducted from October 2008 until May 2009 at the NICU of Lucile Packard Children's Hospital (LPCH). We enrolled premature infants (between 25 and 36 weeks gestational age at birth), due to be discharged at a chronological age of 6–14 weeks. Exclusion criteria were failure to thrive, malignancy, immunocompromised state or receiving immunosuppressive therapy within the past week, hypersensitivity to any component of the vaccine, history of necrotizing enterocolitis, short gut, or malabsorption disorders. The study was approved by the Stanford University Administrative Panel on Human Subjects in Medical Research.

Potentially eligible subjects were identified by screening the NICU patients for the inclusion and exclusion criteria. After informed consent was obtained from the parents, the infants were immunized with RV5 on the day of discharge, vital signs were recorded, subjects were observed for 30 min post-immunization, and then discharged home. Parents received a diary card and instructions for recording fever, irritability, vomiting, and/or diarrhea in the participant, a calendar with specimen collection days highlighted, a diaper collection kit with instructions for stool sampling and instructions to record symptoms experienced by HHC (defined as any person(s) living at the same residence as the subject at anytime during the 4 week study period).

After discharge from the NICU, stool specimens consisting of a whole diaper of bulk stool were collected on Monday, Wednesday, and Friday for the 2 weeks post-immunization for a total of 6 specimens (Fig. 1). The diapers were placed in double biohazard bags, and placed in the freezer; samples were then delivered to Stanford and stored at −20 °C until shipped on dry ice to Cincinnati. Parents recorded on the diary card solicited adverse events for the study subject, and reported if any HHC had fever, vomiting, or diarrhea, and submitted the diary card with the last stool sample. If an HHC had symptoms, a stool sample was to be obtained for rotavirus antigen testing. One of the study investigators (CKS) contacted the parents three times during the 4 weeks after immunization to give reminders for specimen collection and collect solicited adverse events. Data were entered into a study database for analysis.

2.2. Laboratory analysis

Whole diapers containing the stool samples were sent to the Laboratory for Specialized Clinical Studies at Cincinnati Children's Hospital for analysis for presence of rotavirus antigen by enzyme immunoassay (EIA). The EIA used to detect rotavirus antigen in stool samples in this study has previously been described [12]. This lab is the central lab used to evaluate RV5 by the manufacturer and the same EIA assay was used in the vaccine trials, including the REST study [8,14]. This is a sensitive assay in an antigen capture EIA format that uses 96-well test plates. For each sample, duplicate positive test wells were coated with capture antibody purified from rotavirus immunized rabbits and duplicate negative test wells were coated with purified antibody from non-immunized rabbits. Captured virus was detected using guinea pig antirotavirus hyperimmune serum. Detection was amplified using a biotinylated secondary antibody and a preformed avidin–biotin horseradish peroxidase complex. Addition of the enzyme substrate yields values that are interpreted as a positive or negative result. A positive control was included on each plate to determine plate validity.

The rotavirus EIA positive samples were quantified using a standard plaque assay by cultivation in MA104 cells by the same methods used for the REST study [15]. Plaques were picked and the virus isolated was grown in tube cultures of MA104 cells. Cultures were frozen, thawed and an aliquot of the viral lysate was used to isolated the double stranded RNA (dsRNA) by using a QIAamp® Viral RNA Mini Spin Kit (Qiagen, Cat. #52904) according to the manufacturer's instructions. The isolated dsRNA was then run on