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Incidence of rotavirus gastroenteritis hospitalizations and genotypes, before and five years after introducing universal immunization in Israel

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ABSTRACT

Background: Uncertainty exists about the sustainability of the reduction in rotavirus gastroenteritis (RVGE) following the introduction of rotavirus vaccines into national immunization programs, and on its potential impact on circulating genotypes. RotaTeq was introduced into the Israeli national immunization program in December 2010, and vaccination coverage is around 80%.

Aims: To examine the change in incidence of RVGE hospitalization and rotavirus genotypes, during the five years after introduction of RotaTeq into the Israeli national immunization program.

Methods: Data were obtained prospectively on hospitalization of children aged 0–59 months due to acute gastroenteritis (N = 7346) from three hospitals in northern Israel. Stool samples were tested for rotavirus by immunochromatography. Rotavirus was genotyped (N = 506) by RT-PCR and/or sequencing.

Results: The average incidence of RVGE hospitalization declined by 61.0% (95% CI 49.0–73.4%), from 5.6 per 1000 (95% CI 5.0–6.2) in the pre-universal immunization period (2008–2010) to 2.2 per 1000 (95% CI 1.8–2.5) during the universal immunization period (2012–2015), but yearly fluctuations were still observed.

Results: The most common genotypes in the pre-universal immunization period were G1P[8] (35.3%) followed by G2P[4] (15.5%), G3P[8] (8.8%), G4P[8] (4.3%) and G9P[8] (4.3%), and 19.5% were mixed infections. The dominance of G1P[8] continued into the universal immunization period (48.6%), followed by G3P[8] (21.5%), G9P[8] (15.9%) and G12P[8] (4.7%), while mixed rotavirus infections were no longer detected.

Conclusions: Universal immunization with RotaTeq in Israel was associated a sustained reduction in RVGE hospitalization. It is unclear whether changes in the circulating rotavirus genotypes are due to vaccine-induced selective pressure. Assessment of the long-term impact of rotavirus vaccination on the incidence of rotavirus gastroenteritis and continued strain surveillance is warranted.

1. Introduction

Rotavirus remains the leading cause of severe diarrhea in young children [1,2] and it is a major cause of pediatric morbidity and mortality in developing countries [3]. Current rotavirus vaccines, orally administered, pentavalent RotaTeq (Merck) [4] and monovalent Rotarix (GSK) [5] vaccines, have been accessible since 2006. Clinical trials with these vaccines showed good efficacy, 81–100%, in preventing severe rotavirus gastroenteritis (RVGE) in infants in developed countries [4,5], but showed lower efficacy, 39–59%, in developing countries [6–8]. In 2013, the World Health Organization recommended the use of these vaccines in all national immunization programs [9]. This recommendation is being gradually implemented [10] with rapid uptake of rotavirus immunization in many countries such as Belgium, Austria,

Abbreviations: AGE, acute gastroenteritis; RVGE, rotavirus gastroenteritis; CI, confidence intervals.

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Australia, and the United Sates, where immunization coverage has reached 72–89% [11–15]. The implementation of universal rotavirus immunization has been associated with significant declines in the burden of RVGE and all-cause acute gastroenteritis (AGE) in these settings [12–15].

In Israel, RotaTeq was added to the national immunization program at no cost to parents in December 2010. Coverage with three RotaTeq doses at the national level is estimated to be 80% (personal communication: Dr. Emilia Anis, Israel Ministry of Health).

We have previously shown a 61% decline in the incidence of RVGE hospitalization among children less than two years of age in northern Israel during the first three years of universal immunization, as compared to the pre-universal immunization period [16], as well as a significant decline in clinic visits due to RVGE [17].

Despite impressive declines in the burden of RVGE in Israel and elsewhere, many questions remain unresolved, such as the sustainability of these reductions, and the potential for vaccine-induced selective pressure on rotavirus circulating genotypes following the introduction of universal immunization programs.

Aiming at addressing these questions, we examined the changes in incidence of all-cause GE, RVGE hospitalization, and rotavirus genotypes, in children aged 0–59 months, during the first five years after the introduction of RotaTeq into the Israeli national immunization program.

2. Materials and methods

2.1. Study population and design

A hospital-based surveillance study was conducted between November 2007 and February 2016 in northern Israel. Details of the study design have been published previously [16,18,19]. In brief, the study target population was comprised of children 0–59 months of age residing in the catchment area of three hospitals: Hillel Yaffe in Hadera, Carmel in Haifa, and Laniado in Netanya [18,19]. The pediatric population receiving hospitalization services in these hospitals was estimated at 80–90% of the Hadera sub-district, 25% of the Haifa sub-district and 60–70% of the HaSharon sub-district, respectively. Based on the Central Bureau of Statistics report on population size by age and sub-district [20], the annual average number of children 0–59 months of age served by these hospitals during the study period was estimated at ~65,000.

According to the Universal National Health Insurance Law, implemented in 1995, all Israeli citizens have access to medical care (both outpatient and inpatient services), and access is similar across sub-populations and regions in Israel. Immunization of children with those vaccines included in the national immunization program, including RotaTeq, is primarily performed at maternal and child health clinics, regardless of health insurance.

The sampling frame included children aged 0–59 months who were hospitalized in the three hospital during the study period with diarrhea (three or more watery stools during a 24 h period). Demographic information was collected about each child.

2.2. Laboratory methods

A stool sample was collected from children 0 to 59 months of age hospitalized for acute diarrhea, within the first 48 h of hospital admission. Stool specimens were tested for rotavirus antigen by immunochromatography (Rotavirus Dipsticks, Hylabs Rehovot & Novamed, Jerusalem, Israel) according to manufacturers' instructions. The presence of *Salmonella*, *Shigella* and *Campylobacter* in stool was tested by culture [18].

Rotavirus genotyping: 10% stool suspensions were prepared in 0.9% saline and RNA was extracted from rotavirus positive fecal suspensions. Two methods were used for determining P-types (viral spike protein VP4 genotype) and G-types (viral glycoprotein VP7 genotype). For stool samples collected between 2007 and 2010, P-types and G-types were determined by electrophoresis after using generic primers for RT-PCR followed by hemi-nested-PCR using primers and amplification conditions as previously described [21–24]. For stools collected after 2010, cDNA was prepared with M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and random primers (Cat. no. 48190011, Invitrogen, Carlsbad, CA, USA) using the following conditions: denature RNA at 97 °C for 5 min; place tube on ice; add 15 μl of reaction mix [1 μl RT enzyme, 7 μl 5× buffer, 3.5 μl 40 mM $MgCl_2$, 0.7 μl random primers, 1 μl 10 mM dNTP mix, 0.5 μl RNAse inhibitor (40 U/μl), and 1.3 μl sterile water] and incubate at 37 °C for 60 min). The cDNA was amplified in a separate PCR reaction using AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) and the generic external primers for P-typing and G-typing [21,23]. P-typing hemi-nested amplification conditions were: Add 2 μl of cDNA to 48 μl of a mix containing 4.8 μl 10× Invitrogen Taq buffer, 2.5 μl 50 mM $MgCl_2$, 1 μl of 10 mM dNTP mix, 0.2 μl Invitrogen Taq polymerase, 7 μl primer mix, and 32 μl sterile water; heat to 94 °C for 5 min; then run 30 cycles of 1 min at 94 °C, 2 min at 45 °C, and 1 min at 72 °C, followed by a final elongation at 72 °C for 7 min. G-typing hemi-nested amplification conditions were: Add 2 μl of cDNA to 48 μl of a mix containing 4.8 μl 10× Invitrogen Taq buffer, 2.5 μl 50 mM $MgCl_2$, 1 μl of 10 mM dNTP mix, 0.2 μl Invitrogen Taq polymerase, 9 μl primer mix, and 35.3 μl sterile water; heat to 94 °C for 5 min; then run 30 cycles of 1 min at 94 °C, 2 min at 42 °C, and 1 min at 72 °C, followed by a final elongation at 72 °C for 7 min. Both strands of the amplicons were sequenced as described [25]. Sequences were corrected using the Sequencher program version 5.2 (Genecodes, Anne Arbor, Michigan, USA) and genotyped against rotavirus prototypes with Sequencher program and confirmed using BLAST to search the DDBJ/EMBL/GenBank sequence database and by using the RotaC on-line tool for genotyping rotaviruses [26]. Mixtures were confirmed by sequencing using type specific primers [21–24].

2.3. Statistical methods

The incidence (per 1000) and 95% confidence intervals (CIs) of all-cause GE and RVGE hospitalization were calculated by year and by period: prior to the introduction of universal rotavirus immunization (2008–2010) and for the universal vaccination period (2011–2015). RotaTeq was introduced into the national immunization program in December 2010, therefore, we considered 2011 as a transitional year, and in the primary analysis, we compared the incidence between the periods 2012–2015 versus 2008–2010. The reduction in all-cause GE hospitalization and the rates of RVGE hospitalization for the two periods were calculated.

Since rotavirus testing differed significantly ($P < 0.05$) by age group (less in older children), and by year and month of admission (less in the summer; June to August) [16], a weighted analysis was applied as previously described [16] in order to avoid over-representation of rotavirus-positive children. The weights were determined as the inverse of the rotavirus testing fraction (equivalent to sampling fraction), in each age group (0–11, 12–23 and 24–59 months), and by month and year (January 2008 until February 2016).

The Cochrane-Armitage test was used to examine the linear trend in annual, all-cause GE and RVGE hospitalization from 2008 to 2015 as well as the linear trend in percentage of fecal samples positive for any of the three bacterial pathogens.

A two-sided $P < 0.05$ was considered statistically significant. Multiple comparisons, when made, were adjusted using Tukey's procedure.

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