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Rotavirus genotypes circulating in Ontario, Canada, before and after implementation of the rotavirus immunization program

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ABSTRACT

Background and objectives: Ontario introduced a universal publicly-funded group A rotavirus (RVA) immunization program in August 2011, using monovalent vaccine. RVA immunization programs have decreased the incidence of RVA acute gastroenteritis in many countries but it is unclear if it will contribute to the emergence of certain genotypes. We monitored RVA trends and genotypes in Ontario before and after implementation of the publicly-funded immunization program.
Methods: RVA detection was conducted at Public Health Ontario Laboratories from January 2009 to December 2011 (pre-program period) and January 2012 to October 2015 (publicly-funded RVA immunization program period) and number of RVA-positive specimens and percent positivity were analysed. A convenience sample of RVA-positive stool specimens, from September 2010 to December 2011 (pre-program period) and January 2012 to June 2013 (program period), were genotyped using heminested PCR. A literature review on the burden of illness from emergent genotype was performed.
Results: Stool specimens showed a significant decrease in RVA percent positivity from the 36 month pre-program period (14.4%; 1537/10700) to the 46 month program period (6.1%; 548/9019). An increase in the proportion of RVA G10 among genotyped specimens, associated with five different P genotypes, from the pre-program (6.3%; 13/205) to the program (31.5%; 40/127) period was observed. Our literature review identified approximately 200 G10-positive human stool specimens from 16 different countries.
Conclusions: This study documented a decrease in the number of RVA-positive specimens and percent positivity after implementation of the immunization program. An unexpected increase in the proportion of RVA G10 was detected following program introduction. Ongoing RVA surveillance is important in evaluating both the long-term impact of immunization and emergence of RVA genotypes.

1. Introduction

Group A rotavirus (RVA) is a common cause of acute gastroenteritis (AGE) in young children worldwide. Rotavirus genus is made up of dsRNA viruses within the *Reoviridae* family and includes multiple species of rotavirus (Rotavirus A to H) [1]. RVA can infect humans, mammals and birds. It displays significant biodiversity due to acquisition of mutations and more complex evolutionary mechanisms such as genetic reassortment or genomic rearrangement [2]. Molecular epidemiological surveillance of RVA is typically performed by inspecting differences in nucleotide sequences of genes encoding two antigenic proteins on the outer capsid: VP7 for genotype G (glycoprotein) and VP4 for genotype P (protease-sensitive) [2]. The burden of AGE caused by RVA has decreased since the implementation of immunization programs in many countries [3,4]. Annual RVA deaths in children under five years old worldwide was estimated to have decreased from 528000 in 2000 to 215000 in 2013 [5].

Two live, attenuated oral anti-RVA vaccines were licensed by numerous regulators, including Health Canada, and have been included in immunization programs throughout the world. They have been studied and approved for use in young infants. Infants

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and young children are at higher risk of severe AGE since they often have not been exposed to RVA and are also more vulnerable to dehydration [6]. Attenuated monovalent G1P[8] human RVA vaccine (RV1) (Rotarix®, GlaxoSmithKline Biologicals, Belgium) is provided as two doses, scheduled at two and four months of age [7]. Pentavalent human-bovine reassortant vaccine (RV5) contains G1, G2, G3, G4 and P1A[8] antigenic specificities (RotaTeq®, Merck Inc., USA) and is given as three doses at two, four and six months of age [8]. Health Canada authorized the use of RotaTeq® and Rotarix® in August 2006 and October 2007, respectively [9,10]. The province of Ontario subsequently implemented a publicly-funded RVA immunization program in August 2011, providing RV1 at two and four months of age. The RVA vaccine coverage in Ontario was previously estimated to be low before the immunization program was introduced, less than 15% from January 2008 to July 2011 [11]. Full RVA immunization series coverage in the province was estimated to be 73%, 78% and 84% in the first three years of the public-program period [12]. A recent study conducted in Ontario, Canada, showed reductions of 68% in emergency department visits and up to 79% in hospitalizations due to RVA [11]. Multiple randomized control trials have shown variable vaccine efficacies of RV1 and RV5 vaccines against numerous genotypes, including genotypes not present in the vaccines. The cross-immunity for genotypes not found in the vaccines is continuously under evaluation [13]. In 2015, a US study estimated that RV1 and RV5 vaccine effectiveness were both 80% and had heterologous protection against different genotypes [3].

We aimed to study the trends of confirmed RVA-positive stool specimens submitted for testing to Public Health Ontario Laboratory (PHOL) for three seasons before and four seasons after the implementation of the RVA immunization program in Ontario. We monitored the change in RVA detection prevalence, and the changing profile of RVA genotypes before and after the implementation of the immunization program in Ontario.

2. Materials and methods

2.1. Study setting and specimen collection

PHOL is Ontario's reference microbiology laboratory, and provides a large proportion of primary gastrointestinal virology testing for patients in various healthcare settings including community/ambulatory practices, hospitals and other institutions. PHOL also provides testing for gastroenteritis outbreaks in Ontario when these are declared by the local public health units. All stool specimens collected from patients by submitters across Ontario and sent to PHOL for RVA testing as part of routine clinical service between January 2009 and October 2015 were included. The pre-publically-funded RVA immunization program period was called the pre-program period and defined as January 2009 to December 2011 (spanning 36 months). The program period was defined as January 2012 to October 2015 (spanning 46 months). The start of the program period (January 2012) was set five months after the implementation of the immunization program (August 2011) to allow infants to receive two doses, at two and four months old [10]. We obtained demographic information from the laboratory requisition; patient charts were not available for review as PHOL is the provincial reference laboratory and as such has no direct access to patient records. As date of specimen collection was not always provided on the laboratory requisition, we used the date when the specimen was received at PHOL for this analysis. Data were extracted from the PHOL information system for the period January 2009 to October 2015. RVA positive specimens and percent positivity over time and characteristics of patients by age, sex, and settings were analyzed. We also compared the number of gastrointestinal outbreak specimens submitted for testing, and number and percent of outbreaks that were RVA-positive during the pre-program and program periods. In Ontario, a gastrointestinal outbreak is defined as three or more cases showing signs and symptoms compatible with infectious AGE in a specific unit or floor within a four-day period or three or more units/floors having a case of infectious AGE within 48 h [14]. PHOL relies on local public health units to declare gastrointestinal outbreaks as we do not have any means to verify if an outbreak meets the case definition.

2.2. Laboratory testing for rotavirus, including genotyping

PHOL conducts RVA testing using electron microscopy (EM), and/or immunochromatographic testing (ICT) with commercial RVA antigen detection kits [RIDAQUICK Rotavirus (Phoenix Airmid Biomedical Corp., Oakville, Ontario, Canada until October 31, 2011), or Rotascreen (Microgen Bioproducts Ltd, Camberley, UK, since November 1, 2011)] [15]. Identification of RVA-positive specimens by ICT and/or EM was confirmed using a triplex rotavirus/adenovirus/MS2 in-house reverse transcription real-time PCR (RT-rtPCR) assay as previously described before proceeding to genotyping [16,17]. This in-house RT-rtPCR was previously found to have 100% positive and negative agreement compared to the Seeplex Diarrhea-V ACE (Seegene, Seoul, South Korea) [16,17].

A convenience sample of RVA-positive stool specimens collected in children and adults from September 2010 to December 2011 (during the pre-program period, spanning 16 months) and January 2012 to June 2013 (during the program period, spanning 18 months) were genotyped. The convenience sample was based on the availability of residual specimen at the time we conducted the retrospective study, and the representativeness was assessed based on patient's age, region, season and year. Nucleic acid was extracted from diluted stool specimens. Briefly, approximately 100 mg of stool specimen was diluted in 1 mL of PBS, vortexed vigorously and centrifuged at 8000*g* for 10 min. Supernatant was removed and subjected to automated nucleic acid extraction using NucliSENS® easyMag® (bioMérieux, Marcy L'Étoile, France) as recommended by the manufacturer. Reverse transcription PCR (RT-PCR) amplification was performed using Qiagen one step RT-PCR kit (Qiagen, Frederick, MD) and amplification on Bio-Rad thermocylers (Bio-Rad Laboratories, Hercules, CA). Genotyping multiplex heminested PCR was used as previously described [18–25]. Briefly, for G typing, the VP7 gene was reverse transcribed and amplified by using the consensus primers VP7F and VP7R to generate an 881-bp fragment in length. Then, the G type was determined by using different pools of primers reported to be specific for human G types (G1, G2, G3, G4, G8, G9 and G10). For P typing, the consensus primer pair Con2-Con3 was used to generate an 876-bp fragment of VP4, and the P type was determined by using different pools of primers reported to be specific for human P types (P4, P6, P8, P9, P10 and P11). The geographical provenance of emergent RVA genotype specimens collected in Ontario was aggregated based on the regional PHOL (among the 11 PHOL sites across Ontario) where the specimen was submitted for testing. Maps of Ontario were constructed with the geographic information system QGIS (v2.14-Essen, http://www.qgis.org/).

2.3. Statistical analysis

Statistical analyses were performed using Stata/SE version 10.0 (StataCorp LP, College Station, TX, USA). A chi-square test was used to compare patients in terms of demographics, results and settings. Fisher's exact test was used for smaller sample sizes. For the purpose of this investigation, RVA-positive specimens were considered those specimens that were positive for RVA by any laboratory

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