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Original article

Clinical severity and molecular characteristics of circulating and emerging rotaviruses in young children attending hospital emergency departments in France

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ABSTRACT

Group A rotavirus (RVA) is the leading cause of acute gastroenteritis in young children worldwide. A prospective surveillance network has been set up to investigate the virological and clinical features of RVA infections and to detect the emergence of potentially epidemic strains in France. From 2009 to 2014, RVA-positive stool samples were collected from 4800 children <5 years old attending the paediatric emergency units of 16 large hospitals. Rotaviruses were then genotyped by RT-PCR with regard to their outer capsid proteins VP4 and VP7. Genotyping of 4708 RVA showed that G1P[8] strains (62.2%) were predominant. The incidence of G9P[8] (11.5%), G3P[8] (10.4%) and G2P[4] (6.6%) strains varied considerably, whereas G4P[8] (2.7%) strains were circulating mostly locally. Of note, G12P[8] (1.6%) strains emerged during the seasons 2011–12 and 2012–13 with 4.1% and 3.0% prevalence, respectively. Overall, 40 possible zoonotic reassortants, such as G6 (33.3%) and G8 (15.4%) strains, were detected, and were mostly associated with P[6] (67.5%). Analysis of clinical records of 624 hospitalized children and severity scores from 282 of them showed no difference in clinical manifestations or severity in relation to the genotype. The relative stability of RVA genotypes currently co-circulating and the large predominance of P[8] type strains may ensure vaccine effectiveness in France. The surveillance will continue to monitor

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the emergence of new reassortants that might not respond to current vaccines, all the more so as all genotypes can cause severe infections in infants. **A. de Rougemont, CMI 2016;22:737.e9–737.e15**

Introduction

Group A rotavirus (RVA) is the leading cause of acute gastroenteritis in young children worldwide, and is estimated to cause around 453 000 deaths every year in children <5 years old, mostly in developing countries [1]. Although fast and appropriate care has considerably reduced mortality in industrialized countries, rotaviruses are still responsible for considerable morbidity in infants and generate significant health costs.

RVAs belong to the *Reoviridae* family and possess a genome composed of 11 dsRNA genomic segments encoding six structural (VPs) and five/six non-structural (NSPs) proteins. On the basis of the outer capsid proteins VP7 and spikes VP4, RVA can be classified into G and P genotypes, respectively. Nucleotide differences in these two genes currently allow the classification of RVA into 27 G genotypes and 37 P genotypes, among which 12 G and 15 P are associated with infections in humans, hence showing a considerable diversity of strains [2,3]. Both viral outer layer proteins elicit the production of neutralizing antibodies in the host, but no precise G or P type clearly correlates with the severity of the disease. The five RVA genotype combinations G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] are responsible for approximately 90% of RVA infections in children [4]. Of note, uncommon G types such as G5, G8, G10 and G12 have emerged in various areas of the world, notably in tropical regions [5,6].

Local data on the current burden of rotavirus disease, including both virological and clinical aspects, are important for decision-making and optimization regarding immunization strategies [7]. Hence, knowledge of the molecular epidemiology and antigenic diversity of co-circulating rotaviruses is necessary to ensure the suitability and efficacy of vaccines. Indeed, RVA diversity is constantly generated by positive selection of single amino acid mutations in defined epitopes, and particularly in highly divergent regions of the outer capsid protein VP7 [8]. Programmes to monitor rotavirus antigenic drifts that might be caused by specific immunological pressures and to monitor potential reassortments between human or human and animal strains have to be carefully developed.

In France, rotavirus infections occur mostly during the winter and spring seasons. Although deaths remain exceptional, RVA are responsible for about 300 000 acute diarrhoea episodes, half of which are severe, 140 000 consultations and 18 000 hospitalizations each year [9]. In spite of the introduction of RVA vaccines in 2006, coverage remains particularly low in France and was estimated at around 8.9% in French infants <12 months old in 2011 [10]. Since the establishment of the French rotavirus surveillance network by the National Reference Centre for Enteric Viruses (Dijon, France), it has become possible to assess the nationwide circulation of RVA strains, especially in infants. This prospective study was designed to monitor and characterize rotavirus infections, including both viral and clinical data, in children <5 years old suffering from community-acquired acute gastroenteritis and attending paediatric emergency units during the rotavirus vaccine era in France. Special attention was paid to the detection of uncommon strains and emerging reassortants.

Methods

After its approval by the local ethical committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Bourgogne, Dijon, France, on 24 November 2005), the surveillance study was conducted during five consecutive seasons from July 2009 to June 2014, and involved 4800 children <5 years old suffering from rotavirus-induced acute gastroenteritis and attending the paediatric emergency units of 16 French Hospitals in 13 regions, including Paris.

Acute gastroenteritis was defined by at least three soft or liquid stools or three bouts of vomiting in 24 h. Children presenting with chronic diarrhoea, immune deficiency, inflammatory disease of the digestive tract, or nosocomial infections were excluded.

Clinical data, including personal identification and clinical symptoms, were collected from children presenting to paediatrics departments. Informed consent was obtained for all participants. Disease severity was calculated using the Vesikari scale, a 0–20 point numerical score that assesses the clinical severity of rotavirus infections (where higher scores indicate greater severity), as previously described [11]. An episode of gastroenteritis with a score $\geq$11 is considered a severe episode.

The stool samples were routinely screened for RVA using mainly immunochromatographic tests or enzyme immunoassays. All rotavirus-positive samples were stored at −20°C until genotyping by the National Reference Centre for Enteric Viruses, University Hospital of Dijon, France. The rotavirus strains were genotyped using RT-PCR according to the EuroRotaNet methods (www.eurorota.net/docs.php) and using the Qiagen OneStep RT-PCR kit (Qiagen, Hilden, Germany). The VP7, VP4, VP6 and NSP4 PCR products from strains of interest were sequenced with the same primers as for amplifications. All the sequencing reactions were performed using the ABI® PRISM® Terminator Cycle Sequencing Kit on a 3130XL DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences were edited and genotyped using BioNumerics software (Applied Maths NV, Sint-Martens-Latem, Belgium) and a selection of sequences from rotavirus reference strains available from the GenBank database. Phylogenetic analysis was performed using MEGA6 software [12]. After sequence alignment using the MUSCLE program [13], a phylogenetic tree was inferred using the Maximum Likelihood method based on the Tamura three-parameter model, which was the best-fit DNA substitution model for the nucleotide data set submitted. Bootstrap values were calculated for 1000 replicates. The nucleotide sequences of representative G12 strains from this study have been deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) under the following accession numbers: KU291317 to KU291349.

Statistical analyses were performed using STATA® v11.0 software from StataCorp (College Station, TX, USA). Comparisons of clinical data and severity scores between G1 and G12 RVA, and between all RVA genotypes were performed using the Fisher-exact test for categorical data and the Kruskal–Wallis test for quantitative data. Fractional polynomials were used to model the relationship between children's ages and severity scores using a linear regression model including a non-zero intercept. Values of $p \leq 0.05$ were considered significant.

Results

From July 2009 to June 2014, 4800 stools specimens were collected with a mean rate of 73.8 samples per centre and per