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Higher proportion of G2P[4] rotaviruses in vaccinated hospitalized cases compared with unvaccinated hospitalized cases, despite high vaccine effectiveness against heterotypic G2P[4] rotaviruses

J. Matthijnssens[1,†], M. Zeller[1,†], E. Heylen[1], S. De Coster[1], J. Vercauteren[1], T. Braeckman[2], K. Van Herck[2,3], N. Meyer[4], J.-Y. Pirçon[4], M. Soriano-Gabarro[5], M. Azou[6], H. Capiau[7], J. De Koster[8], A.-S. Maernoudt[9], M. Raes[10], L. Verdonck[11], M. Verghote[12], A. Vergison[13], P. Van Damme[2], M. Van Ranst[1] on behalf of the RotaBel study group*

1) *Laboratory of Clinical and Epidemiological Virology, KU Leuven, Leuven,* 2) *Centre for the Evaluation of Vaccination, University of Antwerp, Antwerp,* 3) *Public Health Department, Ghent University, Ghent,* 4) *GlaxoSmithKline Vaccines, Wavre,* 5) *Bayer Schering Pharma, Berlin, Germany (formerly GSK Biologicals),* 6) *Paediatric Department, AZ Damiaan, Oostende,* 7) *Paediatric Department, AZ St Lucas, Ghent,* 8) *Paediatric Department, ZOL Campus St Jan, Genk, Belgium,* 9) *Paediatric Department, Clinique Saint-Pierre, Ottignies,* 10) *Paediatric Department, Jessa Hospital, Hasselt,* 11) *Paediatric Department, AZ Alma, Eeklo,* 12) *Paediatric Department, CHR Namur, Namur and* 13) *Infectious Diseases Unit, University Hospital for Children, Brussels, Belgium*

Abstract

The overall vaccine effectiveness of the monovalent rotavirus vaccine in an observational, prospective, multicentre, hospital-based case–control study in Belgium (RotaBel) was 90%. However, rotavirus genotype and co-infecting pathogens are important parameters to take into account when assessing vaccine effectiveness. In this study we specifically investigated the effect of rotavirus genotypes and co-infecting pathogens on vaccine effectiveness of the monovalent vaccine. In addition, we also investigated the effect of co-infecting pathogens on disease severity. From February 2008 to June 2010 stool samples of rotavirus gastroenteritis cases of a random sample of 39 Belgian hospitals were collected and subsequently genotyped. Fisher's exact tests were performed to investigate the relationships between rotavirus genotype, co-infecting pathogens and disease severity. The vaccine effectiveness of a full series of the monovalent rotavirus vaccine against hospitalized rotavirus gastroenteritis caused by G1P[8] rotavirus strains was 95% (95% CI 77.5–98.7). Against G2P[4], the vaccine effectiveness was 85% (95% CI: 63.7–93.8). G4P[8]- and G3P[8]-specific vaccine effectiveness was 90% (95% CI 19.2–98.7) and 87% (95% CI −5.2 to 98.4), respectively. A post-hoc analysis showed that the genotype distribution was significantly related to the vaccination status ($p < 0.001$), whereby G2P[4] strains were proportionally more prevalent in vaccinated cases than in unvaccinated cases. No statistical associations were found between co-infection status and vaccination status, Vesikari severity score or rotavirus genotype. The high vaccine effectiveness against the individual genotypes implies robust protection of the monovalent rotavirus vaccine against hospitalized rotavirus gastroenteritis caused by the major human rotavirus genotypes. The prevalence of G2P[4] requires continued monitoring.

Keywords: Co-infection, disease severity, G2P[4], rotavirus vaccine effectiveness, selective pressure
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Corresponding author: J. Matthijnssens, Laboratory of Clinical & Epidemiological Virology, Department of Microbiology & Immunology, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium
E-mail: jelle.matthijnssens@uz.kuleuven.ac.be

*Filip Adriaens, Bert Beulens, André Bochner, Johan Colpaert, Jean De Bock, Marie-Laura Gielen, An Heyneman, Marianne Michel, Inge Matthijs, Louis Oosterlynck, Michel Pletincx, Ilse Ryckaert, Annick Sauvage, Emmi Van Damme, Ilse Vlemincx, Philippe Watillon

†These authors contributed equally to this work

Introduction

Globally, rotavirus is the most common cause of severe acute gastroenteritis in infants and young children [1]. The two outer capsid proteins, VP4 and VP7, are used in a dual classification system, where VP4 determines the P-type and VP7 determines the G-type. Although 27 G-types and 37 P-types are currently known, only a few G- and P-genotype combinations (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8]) substantially contribute to the burden of disease in humans [2,3].

Comparison studies using RNA–RNA hybridization assays or complete genome sequencing of all 11 rotavirus gene segments have revealed the existence of two major genotype constellations, represented by reference strains Wa and DS-1 [4,5]. The majority of human P[8] rotaviruses possess a Wa-like genotype constellation sharing highly similar gene segments for the majority of their genome (except for the VP7 gene segment) and are (partially) homotypic to the Rotarix™ G1P[8] vaccine strain. The DS-1-like genotype constellation, containing human G2P[4] rotaviruses, are distantly related to the Wa-like rotaviruses with the majority of their gene segments sharing <85% nucleotide identity and are therefore fully heterotypic to the Rotarix™ G1P[8] vaccine strain [4]. Many epidemiological studies conducted worldwide have revealed strong temporal and geographical genotype fluctuations [6]. Currently, the factors causing these fluctuations are largely unknown, although immunity present in a population due to previous virus exposure, and unknown stochastic variables are likely to play an important role [7].

Recently, two live-attenuated oral rotavirus vaccines, a monovalent human rotavirus vaccine (Rotarix™; GlaxoSmithKline Vaccine, Rixensart, Belgium) and a pentavalent human–bovine reassortant rotavirus vaccine (RotaTeq™; Merck & Co, Whitehouse Station, NJ, USA), have been licensed in many countries worldwide. The monovalent vaccine is based on the attenuated human Wa-like G1P[8] rotavirus strain 89-12 isolated in Cincinnati, Ohio, USA in 1988 [8]. The pentavalent vaccine contains five human–bovine reassortant rotavirus strains containing the human components G1–G4, and P1A[8] [9]. Both vaccines were shown to be highly effective against severe rotavirus gastroenteritis in large clinical trials in developed countries [10–14]. The vaccine efficacy was somewhat lower and more variable in developing countries where phase III clinical trials have been recently conducted for the monovalent (South Africa and Malawi) and pentavalent (Ghana, Mali, Kenya, Bangladesh, and Vietnam) vaccines. In these settings, the vaccine efficacy ranged from 17.6% to 81.5% [15,16]. The WHO recommended inclusion of rotavirus vaccination in national immunization programmes worldwide [17]. Belgian authorities made a recommendation for rotavirus vaccination in October 2006 and partial reimbursement (with a co-payment by parents of €10 per dose) has been installed since November 2006, making Belgium the first EU country to introduce rotavirus vaccines into the routine infant immunization schedule [18]. Based on vaccine sales figures, a vaccine coverage of about 90% (80–85% monovalent vaccine) was quickly reached at the start of the 2007–2008 rotavirus season and maintained in Belgian infants [18–20]. Impressive declines in the rotavirus disease burden were observed after rotavirus vaccine implementation in Belgium [18,19], and several other countries, including Australia [21], Austria [22], Brazil [23], Mexico [24], Nicaragua [25] and the USA [26].

Previously, we have determined the overall vaccine effectiveness of the monovalent vaccine in Belgium [27]. In this paper we describe the genotype-specific vaccine effectiveness from the above-mentioned study, together with the rotavirus genotype distribution per vaccination status, and associations between enteric virus co-infections and vaccination status, and enteric virus co-infections and severity of disease. In addition, we also compared the G1P[8] and G2P[4] genotyping results in vaccinated and unvaccinated children in other clinical studies to confirm our findings.

Methods

Study design

Detailed methods on the study design of RotaBel have been described previously [27]. In summary, RotaBel is an observational, prospective, hospital-based, multicentric, matched (by hospital and age) case–control study, designed based on the WHO generic protocol for monitoring the impact of rotavirus vaccination on gastroenteritis disease burden. Controls were non-gastroenteritis patients attending an outpatient clinic at the same hospital in the same time period as the case. For the RotaBel study 215 gastroenteritis cases and 276 controls were enrolled in the 'ATP-confirmed cohort' from February 2008 to June 2010 [27]. ATP-confirmed cases are defined as PCR-confirmed hospitalized rotavirus gastroenteritis cases with at least one matching control. Except for determining the genotype-specific vaccine efficacy, all analyses in this study were conducted using the ATP-confirmed cohort (215 cases), which was supplemented with cases that were partially vaccinated with the monovalent vaccine, were vaccinated with the pentavalent vaccine or had an unknown vaccination status (33 cases), resulting in a total number of 248 cases.

To determine the genotype-specific vaccine efficacy we included only case–control pairs that contained either a fully vaccinated or unvaccinated case and at least one fully vaccinated or unvaccinated control. This resulted in 160 case–controls pairs. Sequencing methods were used to determine the rotavirus genotype from hospitalized rotavirus gastroenteritis cases [27]. Co-infections with astrovirus, adenovirus and norovirus were investigated using PCR-based methods as explained below.

Virus detection and genotyping

Viral RNA (rotavirus, norovirus and astrovirus) or viral DNA (adenovirus) was extracted using the QIAamp Viral RNA mini

kit or the QIAamp DNA Blood Mini Kit (Qiagen/Westburg, Leusden, the Netherlands) respectively, according to the manufacturer's instructions. Extracted dsRNA was denatured at 95°C for 2 min before rotavirus RT-PCR. The RT-PCR was carried out using the Qiagen OneStep RT-PCR Kit (Qiagen/Westburg). Primer pairs and RT-PCR conditions used for the different RT-PCR are shown in the Supporting information (Table S1). The PCR amplicons were purified with the MSB® Spin PCRapace kit (Invitek, Berlin, Germany), and sequenced using the dideoxynucleotide chain termination method with the ABI PRISM® BigDye Terminator Cycle Sequencing Reaction kit (Perkin-Elmer Applied Biosystems, Waltham, MA, USA) on an automated sequencer (ABI PRISM™ 3130). The forward primers described in Table S1 were used as sequencing primers. The chromatogram sequencing files were inspected using CHROMAS 2.3 (Technelysium, Brisbane, Australia). The samples were genotyped using BLAST analyses.

Data analysis

The vaccine effectiveness against hospitalized rotavirus gastroenteritis by individual genotypes and their associated 95% CI were determined as described previously [27]. Severity of rotavirus gastroenteritis was determined using the Vesikari severity scale (calculated using only data available up to the visit and not for the full duration of the episode of gastroenteritis) [28]. Overall associations (between rotavirus genotype distribution, distribution of co-infecting pathogens, vaccination status of hospitalized rotavirus gastroenteritis cases and Vesikari scale) were investigated by Fisher's exact test. We also conducted an ad hoc analysis to compare genotype distribution between vaccinated and unvaccinated cases. When an overall significance was detected, pairwise comparisons were conducted using a Bonferroni-corrected Fisher's exact test to identify the source of the observed overall differences. Statistical analyses were performed using SAS 9.2 and SPSS 17.0 statistical software packages. The same statistical analyses were performed on the data available from other published clinical trials of the monovalent vaccine.

Results

Rotavirus genotype distribution

Among the 248 hospitalized rotavirus gastroenteritis cases, G2P[4] was the most prevalent genotype (n = 125, 50.4%), followed by G1P[8] (n = 59, 23.8%), G4P[8] (n = 23, 9.3%), G3P[8] (n = 19, 7.7%) and G9P[8] (n = 16, 6.5%). Other genotypes (G8P[4], G6P[14], G12P[6] and G12P[8]) were only found once (0.4%). In two cases (0.8%) more than one rotavirus strain was detected (Fig. 1). As shown in the Supporting information (Fig. S1), there were only minor changes in the genotype distribution between the three rotavirus seasons (partially) overlapping with the study period.

Vaccine effectiveness against hospitalized rotavirus gastroenteritis cases by individual genotypes

The vaccine effectiveness against hospitalized rotavirus gastroenteritis caused by homotypic G1P[8] rotaviruses in

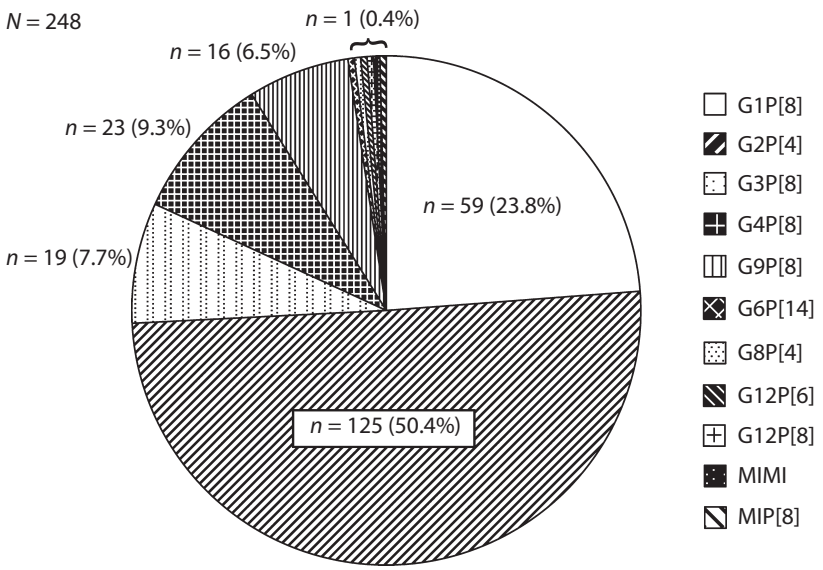

FIG. 1. Distribution of rotavirus genotypes for 248 hospitalized rotavirus gastroenteritis cases. N = number of confirmed rotavirus cases, % = n/number of confirmed rotavirus cases with available results × 100. MI = mixed infection for G- and/or P-genotype.

children receiving a full series of vaccination with the monovalent vaccine compared with unvaccinated children, was 95% (95% CI 78–99%). Against hospitalized rotavirus, gastroenteritis caused by fully heterotypic G2P[4] rotavirus strains, the vaccine effectiveness was 85% (64–94%). G4P [8]-specific vaccine effectiveness was 90% (19–99%) and G3P [8]-specific vaccine effectiveness was 87% (−5% to 98%; Table 1). The vaccine effectiveness against other genotypes could not be determined because of their very low prevalence during the study period.

Rotavirus genotype distribution per vaccination status. The genotype distribution of hospitalized rotavirus gastroenteritis cases according to their vaccination status is shown in Fig. 2 and the Supporting information (Table S2). In the group fully vaccinated with the monovalent vaccine the most prevalent genotype was G2P[4] (68%). In the unvaccinated group, G1P[8] and G2P[4] genotypes were almost equally often found (33% and 32%, respectively). There was a significant overall association ($p < 0.001$) between rotavirus vaccination status and genotype. In particular, when individual genotypes were compared with each other, significant differences were found between the heterotypic DS-1-like G2P[4] strains and the homotypic Wa-like G1P[8] strains ($p < 0.001$), and G2P[4] strains and partially homotypic G4P[8] strains (p 0.004; Fig. 2).

To further investigate this finding, we compared the prevalence of G1P[8] and G2P[4] rotaviruses in vaccinated and unvaccinated cases in other clinical trials with the monovalent vaccine [10–12,14,29–32], using the same methods as in this study (Table 2). Note that the case definition of the rotavirus-positive study subjects for six out of nine trials differed slightly from the present study's case definition.

The distribution of G1P[8] and G2P[4] strains with respect to the vaccination status differed significantly in most studies containing a relatively high number of G1 and G2 cases (>50), such as the studies of Linhares *et al.* [11] (p 0.044), and Ruiz-Palacios *et al.* [14] (p 0.005). Both studies used the same case definition as this study. In the study of Vesikari *et al.* [12]

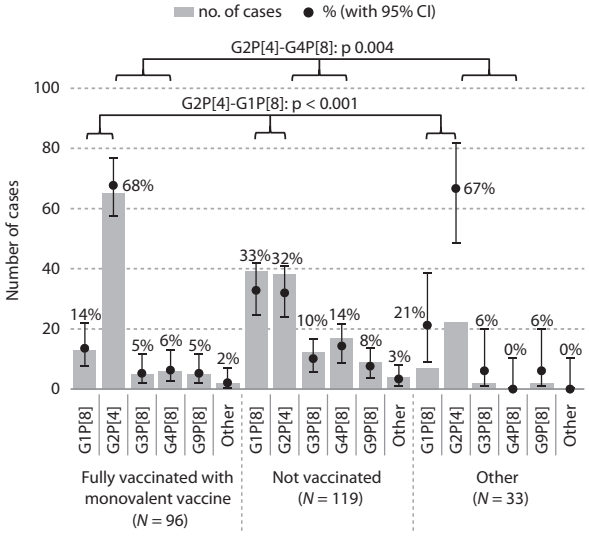

FIG. 2. Statistical analyses of vaccination status per rotavirus genotype for 248 hospitalized rotavirus gastroenteritis cases. Percentages and numbers of the most common human rotavirus genotypes found in rotavirus gastroenteritis patients fully vaccinated with the monovalent vaccine, unvaccinated patients and patients with other vaccination status (partially vaccinated, vaccinated with the pentavalent vaccine, unknown vaccination status) are shown together with their 95% CI. Pairwise comparisons (post-hoc analyses) are indicated by brackets above the bars.

in European infants, a statistically significant difference was also observed in a 2-year study period (p 0.003; Table 2). The study of Li *et al.* [31] conducted in China was the only study, containing a large number of cases infected with either a G1 or G2 rotavirus, were no statistical difference was found between the vaccination status and the distribution of G1 and G2 rotavirus strains (p 0.502). Five studies had a relatively limited number of G1 and G2 cases (<50), including a study conducted in Singapore by Phua *et al.* [10] (22 cases), a study by Kawamura *et al.* [30] in Japanese children (20 cases), a study conducted in Malawi and South Africa by Steele *et al.* [32] (14 and 36 cases, respectively) and a study conducted by Tregnaghi

TABLE 1. Effectiveness against hospitalized rotavirus gastroenteritis of a full series vaccination with the monovalent vaccine by genotype

	Number of cases		Number of controls		
Genotype	**Fully vaccinated***	**Unvaccinated**	**Fully vaccinated[a]**	**Unvaccinated**	**Vaccine effectiveness (95% CI)**
G1P[8]	11	30	48	4	95 (78–99)
G2P[4]	46	34	93	10	85 (64–94)
G3P[8]	4	8	11	2	87 (−5 to 98)
G4P[8]	6	10	15	2	90 (19–99)
All genotypes	70	90	179	19	90 (81–95)

[a]Fully vaccinated means two doses of the monovalent vaccine.

TABLE 2. Statistical comparison of the proportion of G1P[8] and G2P[4] rotavirus strains in cases vaccinated with the monovalent vaccine and unvaccinated cases, in the present study and nine clinical trials ordered according to their total number of cases

	Genotype-specific rotavirus gastroenteritis	Vaccine efficacy/ effectiveness[a] (95% CI)	Monovalent vaccine no. of cases (%)	*Placebo*/Unvaccinated no. of cases (%)	Total number of cases (%)	p-value [b]
Matthijnssens *et al.* (present study)[c]						
Total number of cases:			95	118	213	
	G1	94.67 (77.54–98.74)	13 (13.7)	39 (33.1)	52 (24.4)	**<0.001**
	G2	85.00 (63.69–93.80)	65 (68.4)	38 (32.2)	103 (48.4)	
Vesikari *et al.* 2007 [12][d]						
Combined follow-up period						
Total number of cases:			79	205	284	
	G1	89.8 (82.9–94.2)	18 (22.8)	89 (43.4)	107 (37.7)	**0.003**
	G2	58.3 (10.1–81.0)	14 (17.7)	17 (8.3)	31 (10.9)	
Li *et al.* 2013 [31][d]						
Total number of cases:			70	167	237	
	G1	52.2 (19.0–72.6)	20 (28.6)	38 (22.8)	58 (24.5)	0.502
	G2	58.9 (40.5–72.0)	42 (60.0)	102 (61.1)	144 (60.8)	
Linhares *et al.* 2008 [11][e]						
Total number of cases:			30	154	184	
	G1	82.1 (64.6–91.9)	8 (26.7)	53 (34.4)	61 (33.2)	**0.044**
	G2	38.6 (<0–84.2)	5 (16.7)	8 (5.2)	13 (7.1)	
Ruiz-Palacios *et al.* 2006 [14][e]						
Total number of cases:			11	72	83	
	G1	91.8 (74.1–98.4)	2 (18.2)	34 (47.2)	36 (43.4)	**0.005**
	G2	41.0 (−79.2–82.4)	6 (54.5)	9 (12.5)	15 (18.1)	
Steele *et al.* 2012 [32][d]						
Malawi						
Total number of cases:			41	38	79	
	G1	43.7 (−133.1–85.7)	6 (14.6)	5 (13.2)	11 (13.9)	1.000
	G2	6.2 (−5433.1–95.1)	2 (4.9)	1 (2.6)	3 (3.8)	
Steele *et al.* 2012 [32][d]						
South Africa						
Total number of cases:			15	37	52	
	G1	69.8 (32.5–87.1)	11 (73.3)	18 (48.6)	29 (55.8)	0.3839
	G2	91.8 (32.2–99.8)	1 (6.7)	6 (16.2)	7 (13.5)	
Phua *et al.* 2009[10][f]						
Total number of cases:			2	48	50	
	G1	100 (80.8–100)	0 (0.0)	20 (41.7)	20 (40.0)	Could not be
	G2	100 (−431.7–100)	0 (0.0)	2 (4.17)	2 (4.0)	determined
Kawamura *et al.* 2011 [30][g]						
Total number of cases:			14	34	48	
	G1	84.6 (50.0–96.3)	4 (28.6)	13 (38.2)	17 (35.4)	1.000
	G2	74.9 (−382.2–99.6)	1 (7.1)	2 (5.9)	3 (6.3)	
Tregnaghi *et al.* 2011 [29][d]						
Total number of cases:			7	19	26	
	G1	100 (−1844.0–100)	0 (0.0)	1 (5.3)	1 (3.85)	1.000
	G2	75.1 (−378.7–99.6)	1 (14.3)	2 (10.5)	3 (11.5)	

[a]Vaccine efficacy data as determined in the original study, including cases with double infections.
[b]Two-sided p-values (post-hoc analyses) determined by Fisher's exact test between G1 and G2. p-values lower than 0.05 are shown in bold.
[c]Clinical case definition with at least two episodes of vomiting and/or three episodes of diarrhoea within a 24-h period not due to an underlying medical condition and requiring at least one overnight stay with oral or intravenous rehydration therapy (equivalent to WHO plan B or C). Cases with double infections were omitted.
[d]Clinical case definition with at least three episodes of three looser than normal stools within a 24-h period with or without vomiting.
[e]Clinical case definition with at least an episode of diarrhoea (passage of three or more looser than normal or watery stools within a 24-h period) with or without vomiting that required overnight hospitalization or rehydration therapy (equivalent to WHO plan B or C) in a medical facility such as a hospital, clinic or supervised rural health care centre. Cases with double infections were omitted.
[f]Clinical case definition as in [e] and with additional criterion of a score ≥11 points on the 20-point Vesikari-scale.
[g]Clinical case as in [d], but symptoms led to a medical intervention.

et al. [29] in Latin American children (four cases). In all five studies no statistical difference could be detected or determined.

Hospitalized rotavirus gastroenteritis cases and co-infecting pathogens

Co-infecting pathogens per vaccination status. Sixty-one out of 248 (24.6%) rotavirus gastroenteritis cases were co-infected with astrovirus, adenovirus and/or norovirus. Astrovirus, adenovirus and norovirus co-infections were found in 26 (10.5%), 24 (9.7%) and 3 (1.2%) cases, respectively. In addition, one (0.4%) case was found to be co-infected with both astrovirus and norovirus, and another 7 (2.8%) cases with both astrovirus and adenovirus. No association was found between vaccination status and co-infecting pathogen (p 0.33) (Fig. 3 and Supporting information, Table S3).

Co-infecting pathogens per Vesikari severity scale. In all, 139 (56%) patients hospitalized with rotavirus gastroenteritis were classified as severe according to the Vesikari scale (i.e. a score ≥11 points). To investigate whether co-infection with another viral cause of gastroenteritis (astrovirus, adenovirus or norovirus) would result in a more severe clinical manifestation, we compared the viral co-infection status with the Vesikari score. No overall significant difference (p 0.64) was observed between co-infected cases (astrovirus, adenovirus, norovirus,

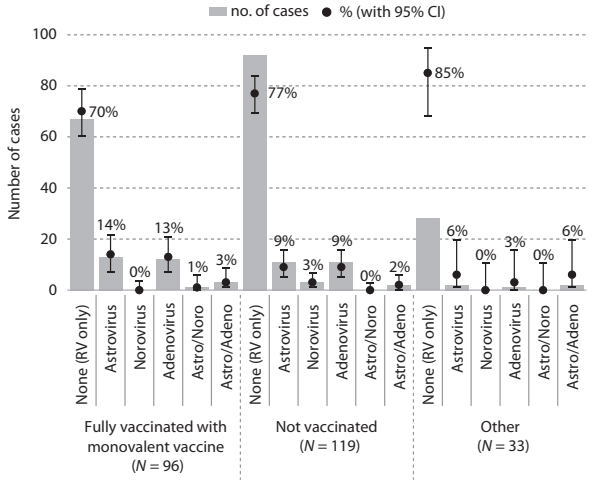

FIG. 3. Statistical analyses of co-infection status by vaccination status for 248 hospitalized rotavirus gastroenteritis cases. Percentages and numbers of patients without co-infection or co-infected with astrovirus, norovirus and/or adenovirus found in rotavirus gastroenteritis cases fully vaccinated with the monovalent vaccine, unvaccinated cases and cases with other vaccination status (partially vaccinated, vaccinated with pentavalent vaccine, unknown vaccination status) are shown together with their 95% CI.

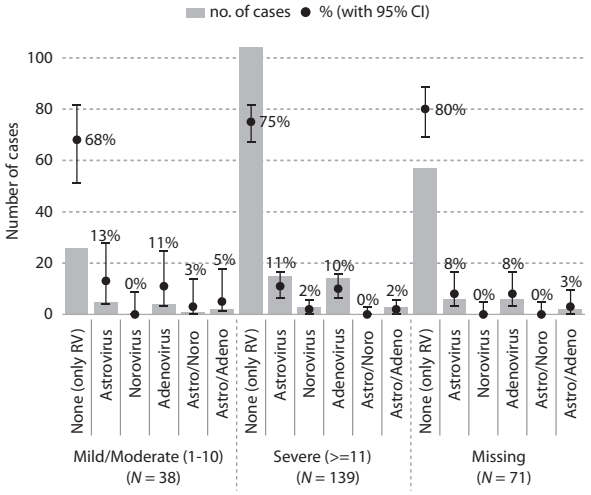

FIG. 4. Statistical analyses of co-infection status versus gastroenteritis disease severity measured by the Vesikari score for 248 hospitalized rotavirus gastroenteritis cases. Percentages and numbers of patients without co-infection or co-infected with astrovirus, norovirus, adenovirus, astrovirus/norovirus or astrovirus/adenovirus found with mild/moderate gastroenteritis, severe gastroenteritis or missing Vesikari severity score are shown together with their 95% CI.

astrovirus/norovirus, or astrovirus/adenovirus) and cases without a co-infection regarding the Vesikari score (mild-moderate vs. severe vs. missing) (Fig. 4 and Supporting information, Table S4).

Co-infecting pathogens per rotavirus genotype. Out of the 61 hospitalized rotavirus gastroenteritis cases co-infected, 43% were G2P[4], 28% G1P[8], 15% G4P[8], 8% G9P[8] and 7% G3P[8] (see Supporting information, Table S5). No significant difference (p 0.78) was observed in the overall distribution of co-infecting pathogens for the different rotavirus genotypes.

Discussion

The study aimed to investigate the rotavirus vaccine effectiveness against different circulating rotavirus genotypes, and to assess the possible effect of co-infections on disease severity and genotype distribution. In a post-hoc analysis we also investigated the genotype distribution per vaccination status.

Natural rotavirus infection or vaccination with a live oral vaccine normally results in immunological responses providing both homotypic and heterotypic protection. This is reflected in a higher vaccine effectiveness of a full series (two doses) of the monovalent vaccine against circulating homotypic G1P[8] rotavirus strains, compared with the circulating heterotypic G2P[4] rotavirus strains—95% (95% CI 77.5–98.7) versus 85% (95% CI 63.7–93.8), respectively (Table 1).

The analysis showed a statistically significant higher proportion of G2P[4] strains in vaccinated cases than in unvaccinated cases. This was also found in the majority of clinical studies for the monovalent vaccine containing >50 G1 and G2 cases (Table 2). Besides the study of Li *et al.* [31] and Steele *et al.* [32], conducted in South Africa, all studies showed a lower point estimate of vaccine effectiveness against rotavirus gastroenteritis caused by the heterotypic G2P[4] strains than against rotavirus gastroenteritis caused by fully homotypic G1P[8], which subsequently translates to a higher prevalence of G2P[4] strains in vaccinated cases compared with unvaccinated cases. In line with this observation, Adlhoch *et al.* [33] showed that during rotavirus surveillance G2P[4] genotypes were more frequently found in breakthrough cases vaccinated with the monovalent vaccine.

From a biological point of view, the finding that the use of vaccines possessing a single or limited number of viral or bacterial strains/types may influence the distribution of co-circulating pathogens in a population is not unexpected, and it is in concordance with the observations of changes in strain/type prevalence after vaccine introduction for other microorganisms, such as human immunodeficiency virus 1 [34] and *Streptococcus pneumoniae* [35] among others.

Strong seasonal and geographical rotavirus genotype fluctuations have also been observed in the absence of vaccines

[6,19]. Currently, the factors causing these fluctuations are largely unknown, although immunity present in a population due to previous virus exposure, and unknown stochastic variables are likely to play an important role. In addition, differences in genotype-specific vaccine effectiveness and the resulting influence on the distribution of co-circulating rotavirus strains, could help to explain the increase in the proportion of G2P[4] strains among the remaining severe rotavirus infections after the introduction of the monovalent vaccine into national immunization schedules, as has been noted in Brazil [36] and to a lesser extent in Australia [37]. Also in Belgium, the strongly decreasing number of rotavirus hospitalizations in three consecutive rotavirus seasons (2006–2009) after vaccine introduction coincided with a higher proportion of G2P[4] rotavirus strains [19]. Unpublished data for the 2009–2013 rotavirus seasons in Belgium indicate that the rotavirus gastroenteritis incidence was between 4.9% and 7.2% of all hospitalized gastroenteritis cases tested for rotavirus and that the proportion of G2P[4] ranged from 16.0% to 64.8% of the remaining rotavirus cases. The latest published and unpublished data from EuroRotaNet do not show a similar prolonged increased proportion of G2P[4] strains in neighbouring countries of Belgium, or any other European countries [6]. This coincides with the fact that Belgium is the only country in Europe where the monovalent vaccine was used for multiple seasons with consistently high vaccination coverage.

To our knowledge, this is the first rotavirus vaccine effectiveness study that also takes into account co-infection with other viral pathogens. To our surprise, no statistically significant association was found between viral co-infections with adenovirus, norovirus or astrovirus and disease severity, indicating that multiple viral infections did not result in a more severe clinical disease manifestation. Also, no association was found between co-infection pathogens and vaccination status, suggesting that vaccine breakthrough cases were most likely not attributable to gastroenteritis caused by co-infecting pathogens. Furthermore no association between co-infecting pathogens and rotavirus genotypes was found, indicating that the higher proportion of G2P[4] strains in the vaccinated population cannot be attributed to an increase in co-infecting pathogens. However, we only screened for the most common viral causative agents of gastroenteritis, while other, mainly bacterial, causative agents could also affect the outcomes of this study.

This is the first European study to estimate genotype-specific effectiveness of a rotavirus vaccine in a post-marketing setting using a robust case–control design and the first to investigate the potential impact of common viral intestinal co-infections on rotavirus vaccine effectiveness [27]. However, this study was not specifically designed to evaluate if rotavirus vaccination may influence the proportion of G2P[4] strains in the rotavirus population. Therefore there is a need for continued longer-term monitoring of the rotavirus genotype distribution in environments with universal rotavirus vaccination programmes to elucidate what the relative importance of vaccination is compared with other factors shaping the yearly changing genotype distribution.

Rotavirus surveillance and detailed strain analyses will remain crucial in the future, because rotavirus strains for which vaccines show a lower vaccine effectiveness might prevail and because new rotavirus variants can be disseminated across the globe in a short time span [2]. Nevertheless, the vaccine effectiveness against hospitalized rotavirus gastroenteritis cases by individual homotypic and heterotypic rotavirus genotypes in Belgium was shown to be high (95% against G1P[8], 85% against G2P[4], 87% against G3P[8] and 90% against G4P[8]), implying robust protection of the monovalent vaccine against hospitalized rotavirus gastroenteritis caused by the major currently circulating human rotavirus genotypes.

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Author Contributions

NM, PVD, MS-G, KVH, JM and MVR designed the study. Clinical staff from GSK contributed on the study set up in all centres. MA, HC, JDK, A-SM, MR, LV, MV, AV and the RotaBel study group were responsible for enrolment of participants and data acquisition. SDC, EH, MZ and JM performed the laboratory analysis. TB was responsible for data acquisition, data management, training and coordination of study staff. MZ,

JV and Statisticians from GSK worked on aspects of the statistical analyses. JM, MZ, PVD, KVH and MVR wrote the manuscript. Publication coordination and editorial management were provided by JM, MZ and GSK. All authors had access to the data used in this paper, contributed to the writing of the manuscript, and have seen and approved the final version.

Transparency Declaration

The Laboratory of Clinical and Epidemiological Virology (MVR, JM, SDC, MZ, EH, JV) received grants from GlaxoSmithKline Biologicals SA, Merck Research Laboratory and Zoetis for rotavirus research. MZ and EH received money for travel and accommodation from GSK for ESPID. ASM received institutional grants from GlaxoSmithKline Biologicals SA. NM and JYP are employees of GlaxoSmithKline group of companies. MR received institutional grants from GlaxoSmithKline group of companies for board membership and lectures and speakers bureau. MSG was an employee of GlaxoSmithKline group of companies between 2005 and 2010 and held stock options. PVD acts as chief and principal investigator for vaccine trials conducted on behalf of the University of Antwerp, for which the University obtains research grants from several vaccine companies, and received payment for lectures which are transferred to an educational fund of the University. MVR received institutional grants from GlaxoSmithKline Biologicals SA, received institutional grants for consultancy with GlaxoSmithKline Biologicals SA, SP-MSD, Johnson & Johnson, and talks at meetings of general practitioners where the meeting was sponsored by GlaxoSmithKline Biologicals SA, Merck, or other companies.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Genotype distribution of hospitalized rotavirus gastroenteritis cases per rotavirus season.

Table S1. Primers used in (RT-) PCR and sequencing protocols.

Table S2. Distribution of status of vaccination by rotavirus genotypes for 248 hospitalized rotavirus gastroenteritis cases.

Table S3. Percentage of rotavirus gastroenteritis hospitalizations attributable to co-infected patients by vaccination status for 248 hospitalized rotavirus gastroenteritis cases.

Table S4. Percentage of rotavirus gastroenteritis hospitalizations attributable to co-infected patients by Vesikari score for 248 hospitalized rotavirus gastroenteritis cases.

Table S5. Distribution of rotavirus genotypes by co-infection status for 248 hospitalized rotavirus gastroenteritis cases.

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