Rotavirus incidence and genotype distribution before and after national rotavirus vaccine introduction in Belgium

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\textbf{Abstract}

Rotarix\textsuperscript{TM} was introduced into the Belgian market in 2006 and RotaTeq\textsuperscript{TM} in 2007, quickly reaching more than 85\% vaccine coverage of all newborns in Belgium. The incidence of rotavirus gastroenteritis has been monitored in the Gasthuisberg University Hospital (GUH), Belgium since 1986, and since 1999 the genotypes of circulating rotavirus strains have been determined. The average percentage of rotavirus positive cases out of all hospitalized gastro-enteritis cases tested (>95\% of these cases are younger than 5 years old) at the GUH between 1986 and 2006 was 19.0\%. This percentage dropped to 12.4\%, 9.6\% and 6.4\% in the three seasons post vaccine introduction (2006–2009), which is a decline of 34.7\%, 49.4\% and 66.3\% respectively. In addition the rotavirus season was found to be shortened and delayed. The prevalence of the G2 genotype sharply increased in the 2006–2007 rotavirus season compared to the previous seasons and remained high (30–40\%) in the 2007–2008 and 2008–2009 seasons. Rotavirus vaccines have strongly reduced the number of children hospitalized due to a rotavirus infection at the GUH; it is however unclear if the predominance of G2 genotypes is related to the vaccine introduction, or if this is attributable to normal genotype fluctuations. Continued surveillance will be pivotal to answer this question in the future.

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\section{Introduction}

Rotaviruses are one of the leading etiological agent of gastroenteritis (GE) in young children, and are estimated to cause more than 600,000 deaths annually worldwide \cite{1}. Rotaviruses belong to the Reoviridae family and possess a genome containing 11 segments of double stranded RNA. The two outer capsid proteins VP7 and VP4 are used for a dual classification system, defining G and P-genotypes respectively. Currently 23 G-genotypes and 32 P-genotypes have been described based on nucleotide sequence variation \cite{2–6}.

In temperate climates, rotavirus infections mainly occur with a seasonal pattern, starting in late autumn and ending in early spring \cite{7,8}. In (sub)tropical areas, rotavirus infections occur all year-round, with or without major peak periods \cite{9}. The rotavirus genotypes G1P[8], G2P[4], G3P[8] and G4P[8] were the most important circulating genotypes in humans worldwide before 1995 \cite{10}. After 1995, the number of countries reporting the emergence of G9 rotaviruses increased dramatically, and G9 is now considered the fifth globally important rotavirus genotype \cite{11}. In addition, G12 is currently also being increasingly detected around the world, and might very well be the next major human genotype \cite{11,12}. Currently, both the G9 and G12 genotypes have shown an epidemiological behavior which is very similar to that of the other major human genotypes G1 to G4. In addition, there is no clear evidence that there might be a correlation between the rotavirus genotype and disease outcome, which could affect hospitalization rates. Although the genotype distribution of these 5 or 6 globally important rotavirus genotypes can change dramatically in a certain region from one year to another \cite{10,13}, or in different geographical regions in the same year \cite{7,14}, the G1P[8] rotavirus genotype remains overall the most prevalent genotype worldwide \cite{7,10}.

The segmented nature of the rotavirus genome allows the occurrence of reassortments resulting in the formation of novel reassortant rotaviruses with genome segments from more than one parental strain. Although numerous examples of such reassortment
are available in literature [12,15–21], certain genome constellations appear to be found preferentially together [22,23]. The large majority of human rotavirus can be assigned to two large distinct genome constellations or genogroups represented by reference strains Wa and DS-1. The G1P[8], G3P[8], G4P[8], G9P[8] and G12P[8] strains do almost always belong to the Wa-like genogroup, whereas G2P[4] strains almost always belong to the DS-like genogroup [2,12,22,23].

Two live, oral rotavirus vaccines, Rotarix™ (GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq™ (Merck & Co., Inc., Whitehouse Station, NJ, USA), are now licensed in many countries around the world. Rotarix™ is based on a live attenuated human G1P[8] rotavirus strain, belonging entirely to the Wa-like genogroup [24]. The pentavale, live and attenuated human-bovine reassortant vaccine, RotaTeq™, is based on a modified Jennerian approach, containing five human genotypes, G1, G2, G3, G4, and P[8], and the bovine genotypes, G6 and P[5], into the backbone of the bovine WC3 strain [25]. Although the gene segments of the bovine backbone of the reassortants in RotaTeq™ do not belong to either of the two typical human genogroups it is believed that the bovine gene constellation has a common origin with human rotavirus belonging to the DS-1-like genogroup [2]. Large-scale Phase III clinical trials have been conducted to show that both vaccines are generally well tolerated and there was no association with intussusception [26–28]. In addition, both vaccines have been shown to be immunogenic and highly efficacious in developed countries and Latin America [24]. Phase III clinical trials have been recently conducted in Ghana, Mali, Kenya, Bangladesh, and Vietnam (RotaTeq™), and in South Africa and Malawi (Rotarix™), to evaluate the safety, immunogenicity, and efficacy of these rotavirus vaccines in Africa and Asia. In these settings the vaccine efficacies ranged between 17.6% and 81.5% [29–32]. The first dose of vaccination is recommended to be administered between the age of 6 and 15 weeks, and the last dose not after the child has reached the age of 8 months, because of insufficient data on the safety of the vaccines outside this age window. Rotarix™ and RotaTeq™ have been commercially available in Belgium since June 2006 and June 2007 respectively, and a routine administration program together with a governmental co-payment policy (adopted in November 2006 for Rotarix™ and June 2007 for RotaTeq™) resulted in a very high vaccine uptake. This co-payment policy pays for approximately 80% of the public price of the vaccines, resulting in only approximately €10 to be paid by the parent per rotavirus vaccine dose [4]. Up to now, mainly Rotarix™ has had a high uptake. Monitoring of children with GE for the presence of rotavirus antigen has been conducted since the 1980s in the Gasthuisberg University Hospital (GUH) in Leuven [33].

This study describes the incidence of rotavirus gastroenteritis (RGE) in the GUH in Leuven, Belgium, from 1986 to 2009 and the genotype distribution between 2003 and 2009, three years before and three years after the start of rotavirus vaccination in Belgium. The rotavirus genotype distribution between 1999 and 2003 has been described previously [13].

2. Materials and methods

2.1. Rotavirus antigen detection

All children under the age of 5 presenting with GE and older people presenting with rotavirus specific symptoms hospitalized in GUH were tested for rotavirus. Rotavirus antigen detection in stool samples from hospitalized GE-cases (both community and hospital acquired) from 1986 to 1995 was performed using the TestPack® Rotavirus (Abbott Laboratories, Chicago, IL), and from 1996 to 2009 using the PremierTM Rotaprime® (Meridian BioScience).

2.2. Rotavirus genotyping

Viral RNA was extracted using the QIAamp Viral RNA mini kit (Qiagen/Westburg, Leusden, The Netherlands) according to the manufacturer’s instructions. The extracted RNA was denatured at 95 °C for 2 min. Reverse transcriptase PCR (RT-PCR) was carried out using the Qiagen OneStep RT-PCR Kit (Qiagen/Westburg) using primers Beg9 and End9 (VP7), and VP4.1–17F and Con2 (VP4) as described previously [13]. Briefly, the reaction was carried out with an initial reverse transcription step at 50 °C for 30 min, followed by PCR activation at 95 °C for 15 min, 35 cycles of amplification (30 s at 94 °C, 30 s at 50 °C for VP7 and 30 s at 45 °C for VP4, 1 min at 72 °C), and a final extension of 10 min at 72 °C in a Thermocycler Biometra T3000 (Biometra, Westburg BV, Netherlands). PCR products were run on a polyacrylamide gel, stained with EtBr and visualized under UV-light. The PCR amplicons were purified with the MSB® Spin PCRapace kit (Invitex, Germany), and sequenced using the dideoxynucleotide chain termination method with the ABI PRISM® BigDye Terminator Cycle Sequencing Reaction Kit (Perkin-Elmer Applied Biosystems, Foster City, CA) on an automated sequencer (ABI PRISM™ 3100). The primers Beg9 and VP4.1–17F described above were used as sequencing primers. The chromatogram sequencing files were inspected using the computer application Chromas 2.3 (Technelysium, Helensvale, Australia). The samples were genotyped using the RotaC v1.1 genotyping tool (http://rotac.regatools.be) [34] or using the National Center for Biotechnology Information (NCBI, National Institutes of Health, Bethesda, MD) BLAST (Basic Local Alignment Search Tool) server on GenBank database.

2.3. Vaccine coverage and seasonality

Rotavirus vaccine administration was estimated based on monthly vaccine sales figures of Rotarix™ and RotaTeq™ in Belgium. Assuming that every child receives a full vaccine regimen (2 doses for Rotarix™ and 3 doses for RotaTeq™), allowed us to calculate the number of children presumably vaccinated. Five-month moving averages were calculated for the estimated number of vaccinated children to smoothen the graph. Monthly birth statistics for the period August 1986–July 2009 were retrieved from the FOD Economy (Federal government). Vaccine coverage was estimated from January 2007 until December 2009 by dividing the number of vaccinated children by the number of newborn children in this period. To compare the seasonality of rotavirus incidence before and after vaccine introduction we used a similar method as was previously applied on rotavirus surveillance data from the US [35,36]. Median, minimum and maximum monthly RGE incidences from seasons before vaccine introduction (1986–2006) together with three seasons post vaccine introduction were plotted. The rotavirus epidemic season was defined as the period during which the percentage of rotavirus positive samples of all tested diarrheal samples was 10% or higher. In case the 10% threshold was in between two months, the intersection between the 10% threshold and a linear extrapolation between those two months was used to determine the onset or the end of the rotavirus season.

3. Results

3.1. Rotavirus incidence from 1986 to 2009

The number of hospitalized GE cases tested for rotavirus antigen, and the number of rotavirus positive cases at the GUH are available since 1986. In Fig. 1A, these data are plotted, together with the percentage positivity rate per season. A rotavirus season in Belgium is defined from August to July. Although there are fluctuations in the
Fig. 1. (A) Number of hospitalized GE cases (green bars) and number of hospitalized GE cases tested rotavirus positive (blue bars) per season at the GUH from 1986–1987 until 2008–2009. The red line indicates the percentage rotavirus positive tested in a particular season. Black dotted lines indicate the vaccine introductions in Belgium. (B) Number of rotavirus positive cases per month at the GUH from 1986–1987 until 2008–2009 (blue). The number of births per month (yellow) and the estimated five-month averaged number of vaccinated children based on vaccine sales figures (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
number of hospitalized GE cases and in the number of rotavirus positive cases over the years before the introduction of vaccination (1986–2006), the percentage of rotavirus positive cases was relatively stable and ranged between 15.2% and 23.1%. However, in the rotavirus seasons following the introduction of vaccination this percentage dropped to 12.4% (2006–2007), 9.6% (2007–2008) and 6.4% (2008–2009). Compared to the average percentage of RV positive cases (19.0%) in 20 seasons before vaccine introduction (1986–2006), the decline in the percentage of RV positive cases was 34.7%, 49.4% and 66.3% in 2006–2007, 2007–2008 and 2008–2009 respectively.

The number of rotavirus cases per month at the GUH together with the number of births in Belgium per month and the estimated number of vaccinated children per month is shown in Fig. 1B. There is a very rapid increase in the number of vaccinated children in the months after the licensing of Rotarix™ in Belgium, due to the governmental co-payment policy and the routine vaccine administration to the majority of all newborns in Belgium. After a steep initial incline in the number of vaccinated children, this number remains relatively stable for the next months. The average vaccine coverage from January 2007 until December 2009 was estimated to be 88.4%.

In Fig. 2, these same data are further broken down, with the median pre-vaccination number of RGE cases per month (from 1999 to 2006) shown as a black line, and the gray shaded area as the minimum and maximum number of RGE cases per month in this period. The three seasons post vaccine introduction (2006–2009) are shown in colored lines, clearly showing a decrease in RGE cases, and an apparent delay in the onset of the rotavirus season of approximately 2.25 months in 2006–2007, 2.5 months in 2007–2008 and 3.25 months in 2008–2009. However, the peak incidence of a rotavirus season was delayed to a lesser extent: 1 month (2006–2007 and 2007–2008) and 1.5 months (2008–2009) compared to seasons before vaccine introduction. In addition, the length of a rotavirus season (based on the RGE positivity rate in hospitalized GE cases) is drastically shortened from almost 7 months before vaccine introduction to less than 2.5 months in the 2008–2009 season.

3.2. Age distribution

The breakdown of the data by age of the RGE patients is shown in Table 1. Although the absolute number of RGE cases dropped from an average of 190 in pre-vaccine seasons to 93 in 2006–2007, the relative distribution of age groups compared to pre-vaccine seasons has not been changed in 2006–2007 despite the fact that only 0–1 year olds were eligible for vaccination, which could suggest a herd immunity effect. However, in 2007–2008 and 2008–2009 there is an apparent decline of GE cases in the 0–1 year old age group (32.3% and 42.5%) compared to seasons before vaccine introduction (1999–2006 average: 59.3%), although this could be an artefact of the decreasing sample size (Table 1).


The rotavirus genotype distribution in the GUH in 4 consecutive rotavirus seasons from 1999 to 2003 has been published previously and described the emergence of the G9 genotype in Belgium (approximately 50% in the 2002–2001 and 2002–2003 seasons), and a notable increase in the prevalence of the G3 genotype in the 2002–2003 rotavirus season [13]. These data together with the new data for the 2003–2006 (before vaccine introduction) and 2006–2009 (post vaccine introduction) seasons are shown in Fig. 3. The increase in the prevalence of G3 in the 2002–2003 season continued in 2003–2004 with a strong additional increase of the G4 genotype. G3 and G4 together were responsible for more than 80% of the RGE cases in 2003–2004, with G1 and G9 each being responsible for less than 10% of the cases (Table 1). The 2004–2005 season was characterized by the re-emergence of G9 (46.2%) and a small increase in the prevalence of G1 (23.7%). The 2005–2006 season was again completely dominated by G1 (73.0%) with the remaining strains being mostly G9 (Fig. 3). In the first season (2006–2007) after the introduction of Rotarix™ (G1P[8]), G2 emerged being responsible for approximately one third of the RGE cases (31.5%). G1 and G9 each also caused almost one third of the RGE cases in the 2006–2007 (both 28.3%) (Fig. 3). G1 and G2 remained high in the 2007–2008 season (44.4% and 36.5% respectively), and G12 reappeared in Belgium after they were first detected in 2003–2004 [12]. The third rotavirus season after the start of vaccination (2008–2009) was characterized by a maintained high prevalence of G2 (38.5%). The remainder of the RGE cases was caused by G1 (15.4%), G9 and the reappearing G3, each being responsible for 20.5% of the RGE cases. Remarkable was also the isolation of a few uncommon G6 strains in the 2005–2006, 2007–2008 and 2008–2009 seasons (Fig. 3).
For the 2007–2008 and the 2008–2009 seasons the P-genotype was also determined. For both seasons P[8] was the most observed genotype followed by P[4] (61.8% and 59% for P[8] and 38.1% and 41.1% for P[4] respectively) (Supplementary data 1). By far most genotypes were found in typical genotype combinations (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8]). Besides these classic genotype combinations, one G6P[4], one G8P[8] and one G9P[4] strain were found.

4. Discussion

Since 2006, both RotarixTM and RotaTeqTM have been licensed in many countries around the world, and the first data on the impact of rotavirus vaccination on the prevalence of disease caused by rotavirus infections are slowly becoming available. Data from the National Respiratory and Enteric Virus Surveillance System and New Vaccine Surveillance Network in the United States revealed a >50% drop in the number of hospitalized children due to rotavirus GE in the 2007–2008 rotavirus season in comparison with the previous 15 seasons spanning 1991–2006 [35]. Recent data from hospitals across the United States indicate that RotaTeqTM has a remarkable drop of 74% in hospitalization due to RGE in the vaccine introduction. With an average vaccine coverage of 72%, a strong reduction in rotavirus GE cases has been reported in several hospital based studies in the United States. However, these reductions (>70%) cannot be exclusively attributed to the direct effect of RotaTeqTM, as the vaccine has an uptake of well below 50% in the United States. In the Austrian study, next to the reduction was found within a few months (Fig. 1B), mainly due to government organized routine vaccine administration in Belgium. This centralized approach allows for a very rapid implementation of changes in the vaccination schedule of newborns. The apparent initial peak and the subsequent decline in the estimated number of vaccinated children (Fig. 1B) probably reflects storage of vaccines by pharmacists after the launch of RotarixTM. Due to a very efficient pharmacy delivery system in Belgium (multiple deliveries per day), pharmacists only have to store small numbers of vaccine doses, and therefore the chance of a vaccine dose to expire is nearly non existing, making the sales figures a very reliable estimate for vaccine administration. The 88.4% vaccine coverage estimate is based on the assumption that all children received a full vaccine regimen, whereas a small number of children might not have received the full regimen. Therefore it is safe to assume that 85–90% of all newborn babies in Belgium are (at least partially) vaccinated against rotavirus GE.

Fig. 2 suggests a delay in the onset of the rotavirus season in the post vaccine introduction period (2006–2009) in Belgium, a phenomenon that has also been observed after introduction of RotaTeqTM in the USA [29,35,36]. As was mentioned above, a very strong reduction in rotavirus GE cases has been reported in several hospital based studies in the United States. However, these reductions (>70%) cannot be exclusively attributed to the direct effect of RotaTeqTM, as the vaccine has an uptake of well below 50% in the United Sates. In the Austrian study, next to the reduction in the number of RGE cases in the vaccine eligible age group, a decrease was also noted in the younger age group (being not vaccinated, or receiving the incomplete vaccination schedule), whereas no reduction was seen in the older age range [45]. These observations suggest that some kind of herd immunity could play a role in decreasing the burden of disease. A potential herd immunity effect was also observed in our study in the 2006–2007 season, where an apparent reduction in the number of children with rotavirus GE was noted in all age groups, and not only in children less than 1 year (eligible for vaccination). This potential herd immunity effect was not observed anymore in the 2007–2008 and 2008–2009 seasons, possibly due to the decreased sample size.

Following up to 3 years after the worldwide introduction of the two rotavirus vaccines, there is no clear evidence yet for vaccine driven immune selection on circulating human rotavirus strains. A recent review by Leite et al. [47] summarizes the molecular G and P genotype distribution of rotaviruses detected in Brazil for 25 years, between 1982 and 2007. In the 2006–2007 rotavirus season, RotarixTM was introduced in Brazil, and this coincided with a high prevalence of G2P[4] strains [48,49]. Although it might be tempting to speculate that the re-emergence of the G2P[4] strains in Brazil was due to the introduction of RotarixTM, it is too early to tell

Table 1
Number of rotavirus positive cases broken down per age group from 1999–2000 until 2008–2009.

<table>
<thead>
<tr>
<th>Year</th>
<th>0–1 year</th>
<th>1–2 year</th>
<th>2–3 year</th>
<th>3–4 year</th>
<th>4–5 year</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999–2000</td>
<td>138 (59.2%)</td>
<td>61 (26.2%)</td>
<td>18 (7.7%)</td>
<td>13 (5.6%)</td>
<td>3 (1.3%)</td>
<td>233 (100%)</td>
</tr>
<tr>
<td>2000–2001</td>
<td>116 (67.8%)</td>
<td>40 (23.4%)</td>
<td>13 (7.6%)</td>
<td>1 (0.6%)</td>
<td>1 (0.6%)</td>
<td>171 (100%)</td>
</tr>
<tr>
<td>2001–2002</td>
<td>101 (56.1%)</td>
<td>51 (28.3%)</td>
<td>15 (8.3%)</td>
<td>9 (5.0%)</td>
<td>4 (2.2%)</td>
<td>180 (100%)</td>
</tr>
<tr>
<td>2002–2003</td>
<td>130 (57.5%)</td>
<td>61 (27.0%)</td>
<td>25 (11.5%)</td>
<td>6 (2.7%)</td>
<td>4 (1.8%)</td>
<td>226 (100%)</td>
</tr>
<tr>
<td>2003–2004</td>
<td>106 (57.0%)</td>
<td>37 (19.9%)</td>
<td>25 (13.4%)</td>
<td>17 (9.1%)</td>
<td>1 (0.5%)</td>
<td>186 (100%)</td>
</tr>
<tr>
<td>2004–2005</td>
<td>105 (62.9%)</td>
<td>38 (22.8%)</td>
<td>12 (7.2%)</td>
<td>9 (5.4%)</td>
<td>3 (1.8%)</td>
<td>167 (100%)</td>
</tr>
<tr>
<td>2005–2006</td>
<td>95 (55.9%)</td>
<td>51 (30.0%)</td>
<td>14 (8.2%)</td>
<td>5 (2.9%)</td>
<td>5 (2.9%)</td>
<td>170 (100%)</td>
</tr>
<tr>
<td>2006–2007</td>
<td>58 (62.4%)</td>
<td>24 (25.8%)</td>
<td>8 (8.6%)</td>
<td>3 (3.2%)</td>
<td>0 (0.0%)</td>
<td>93 (100%)</td>
</tr>
<tr>
<td>2007–2008</td>
<td>20 (32.3%)</td>
<td>25 (40.3%)</td>
<td>13 (21.0%)</td>
<td>2 (3.2%)</td>
<td>2 (3.2%)</td>
<td>62 (100%)</td>
</tr>
<tr>
<td>2008–2009</td>
<td>17 (42.5%)</td>
<td>11 (27.5%)</td>
<td>9 (22.5%)</td>
<td>3 (7.3%)</td>
<td>0 (0.0%)</td>
<td>40 (100%)</td>
</tr>
</tbody>
</table>
Rotaviruses are monitored in a nationwide surveillance network in Australia since 1999 [54]. Both Rotarix™ and Rotaseq™ have been introduced into the National Immunization program of Australia since July 2007, and each state was allowed to choose one of the vaccines [54]. Rotavirus genotype data for 2007–2008 (the first season post-vaccination) show no obvious changes in the genotype distribution [55]. Although G2 and G9 were found more frequently in states using Rotarix™, G3 was found more frequently in states using Rotaseq™. This is probably due to natural genotype fluctuations, as strongly yearly and geographical fluctuations in the prevalence of different genotype have been previously observed in Australia [54].

As Rotarix™ (G1P[8]) mainly has been used in Belgium, we were particularly interested in investigating the prevalence of the G1 and non-G1 genotypes in consecutive rotavirus seasons. Although our Belgian data reconfirm the presence of strong yearly genotype fluctuations, it should be noticed that, since the introduction of the rotavirus vaccines, a significant increase in the prevalence of the G2 genotype has been observed (above 30%), and this remained the case for the 2007–2008 and 2008–2009 seasons (Fig. 3). In contrast to other circulating genotypes however, very low numbers of G2 strains were found in seasons before vaccine introduction (1999–2006) and the prevalence of G2 genotypes never surpassed 20%. Recent data from the EuroRotaNet surveillance system in Europe also showed a significant increase in the prevalence of the G2 genotype from 2005–2006 to 2006–2007 in countries without a mass rotavirus vaccination programme, such as Hungary, France, Slovenia and the UK [56,57]. However in the 2007–2008 and 2008–2009 seasons the G2 prevalence in Belgium remained high whereas the prevalence of G2 decreased again in the other European countries in 2007–2008 [56,57]. This could suggest that the sustained dominance of the G2 genotypes in Belgium is vaccine-related. However, there are currently only limited data available concerning this issue and prolonged monitoring and results from other countries where Rotarix™ is the predominant vaccine are needed for further evaluation.

At first sight the G1 genotype does not seem to be particularly affected by routine vaccination, as the relative frequency of the G1 genotype does not significantly drop or fall outside of the expected regular genotype fluctuations. However, these results should also be interpreted very carefully as it is known that different lineages of G1 rotaviruses can (co-)circulate in the same season, or fluctuate over the course of different rotavirus seasons [58–60]. It is not unthinkable that certain G1 lineages are selected above others due to vaccine pressure. Selective pressure could potentially facilitate genetic drift, resulting in divergent G1 strains which are less affected by the G1 strain in the Rotarix™ vaccine. To further investigate this possibility, merely genotyping is not sufficient. Consequently, more detailed phylogenetic analysis or amino acid analysis will be necessary to detect such subtle changes. In line with this reasoning it is important to mention that most likely not only VP7 and VP4 can be affected by the vaccines. Especially since the correlation between neutralizing antibody titers against VP7 and VP4 and protection in vivo is incomplete, this suggests that other mechanisms of protection play a role and that several other gene segments might be subjected to selective pressure by the vaccines as well. To further investigate this, complete genome analysis of circulating rotaviruses is necessary.

The further evolution of the circulating rotavirus strains in Belgium under a continued presence of vaccines pressure will be monitored very closely. This will be crucial as it was recently calculated for G9 and G12 that new or variant rotavirus strains can spread across the entire world in a period as short as a decade [61].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.vaccine.2010.09.004.

References
