14-year experience of National Reference Center for Rotavirus A in Belgium: unusual dominance of equine-like G3P[8] genotype after the pandemic

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14-year experience of National Reference Center for *Rotavirus A* in Belgium: unusual dominance of equine-like G3P[8] genotype after the pandemic

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Summary

Introduction

Despite vaccine availability, rotavirus persists as a leading cause of gastroenteritis in children under five years of age, and continued surveillance of circulating genotypes is needed to monitor their evolution.

Methods

We collected 8024 rotavirus positive samples throughout Belgium between 2009 and 2023. For 6352 samples, we were able to determine the G and/or P genotypes through sequencing of the genes encoding the outer capsid proteins VP7 and VP4.

Results

In the pre-pandemic period, we received an average of 622 samples per season, which decreased to 114 and 111 samples during the two pandemic seasons. In the first post-pandemic season we observed a peak surge of 1048 samples. Notably, the proportion of cases in the 2-5-year-old age group increased from 20.3% before the pandemic to 33% after the pandemic ($p<0.001$). Over the 14-year study period, the most common genotypes were G2P[4], G3P[8], and G9P[8]. Post-pandemic data show an unusually strong dominance of the "equine-like G3P[8]" genotype in two consecutive seasons (2021-2023). These equine-like G3P[8] strains are strongly associated with the DS-1-like genotype constellation and were first identified in 2014-2015 in Belgium. Additionally, a significant overrepresentation of vaccinated individuals was found within the equine-like VP7 carrying G3P[8]-infected patient samples compared to infections with other genotypes, including typical human VP7 G3P[8].

Conclusion

Despite the presence of typical seasonal genotype fluctuations, the pandemic seems to be associated with several epidemiological changes, including the unusually strong dominance of an emerging rotavirus strain, against which currently used vaccines may be less effective. It is important to continue close monitoring of this strain to investigate whether this is a temporary phenomenon or if these strains may pose an increased public health threat.

Introduction

Rotavirus infections cause a significant economic and clinical burden globally, particularly in children under five years of age. Clinical manifestations include severe diarrhea, vomiting, and fever, often necessitating hospitalization and medical intervention. It was estimated that among children under five years of age, more than 1.7 million hospitalizations occurred due to rotavirus in 2019, despite an estimated five hundred thousand hospitalizations being prevented due to vaccination (1). However, the global estimate of rotavirus yearly deaths in children under five ranges from 122 thousand to 215 thousand (2,3).

Human rotaviruses, belonging to the family *Sedoreoviridae*, genus *Rotavirus* and species *Rotavirus A*, are characterized by a segmented double-stranded RNA genome, enclosed within three concentric capsid layers. The genotypic classification of rotaviruses primarily relies on the outer capsid proteins VP4 and VP7, which define the P and G genotypes, respectively. These proteins are pivotal in eliciting neutralizing antibodies and form the basis for vaccine development (4). Common rotavirus genotypes G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] have been identified globally, with G1P[8] being the most prevalent (5,6). Therefore, Rotarix (containing an attenuated human G1P[8]) and RotaTeq (composed of a combination of five human-bovine reassortant strains possessing the typical human rotavirus genotypes G1, G2, G3, G4, and P[8]) were developed and have been in use since 2006 (7,8). In Belgium, the introduction of the Rotarix vaccine in 2006 and RotaTeq vaccine in 2007 marked a significant milestone in rotavirus prevention, with Rotarix being the predominantly used vaccine, achieving an uptake of over 85% in short time (5).

The segmented nature of the rotavirus genome allows for reassortment among human and/or animal rotavirus strains, resulting in high genetic diversity, which may lead to the emergence of novel genotypes in the human population that may challenge the effectiveness of available vaccines. Additionally, different genotypes are known to co-circulate with great seasonal fluctuations in the absence or presence of vaccination programs (9). However, in several countries, after the implementation of Rotarix, a shift from the dominant G1P[8] genotype towards the heterotypic G2P[4] was reported (5,10–14). When the complete rotavirus genome (11 segments) is considered, most human rotaviruses possess one of the two gene

constellations, often referred to as either a “Wa-like” or “DS-1-like” genotype constellation (15). Generally, the strains G1P[8], G3P[8], G4P[8], G9P[8], and G12P[8] are associated with the Wa-like genotype constellation, whereas the G2P[4] and G9P[4] strains are most often linked with the DS-like genotype constellation (15–20). However, novel “equine-like” G3P[8] rotavirus strains have been detected in recent years, which also carry this DS-1-like genotype constellation (21,22). Despite fluctuations in genotypes, vaccines continue to be effective in preventing severe disease and hospitalizations (23), although the overall efficacy of the monovalent rotavirus vaccine is slightly lower towards fully heterotypic genotypes, including DS-1-like G2P[4] strains (24).

During the COVID-19 pandemic, the authorities of numerous nations, including Belgium, implemented a range of public health measures starting as of March 2020. The measures included mask mandates, school closures or occupancy limits, and comprehensive lockdowns, which presumably impacted transmission of most communicable diseases. Significant decreases in rotavirus infections have been reported, with some countries reporting reductions of up to 83% in cases compared with previous years (25). However, as restrictions eased, a resurgence of rotavirus and other communicable diseases including acute gastroenteritis was observed (26). This resurgence was most likely due to a combination of lifted measures resulting in strongly increased interpersonal contacts and the lack of immune stimulation due to the reduced circulation of microbial agents during the pandemic, sometimes also referred to as “immunity debt” (27). While changes in rotavirus epidemiology in the post-pandemic era remain unclear, continued surveillance including genotype and vaccination information is needed to better understand the implications of the current and future outbreaks on rotavirus circulation and vaccine effectiveness.

This pandemic situation, provided us with a unique “natural experiment”, allowing us to better understand the epidemiology of infectious diseases including rotavirus. In this study, we leveraged data from the National Reference Center (NRC) for rotavirus in Belgium, which has been running for 14 seasons and provides a comprehensive overview of rotavirus infections in Belgium. We aimed to reveal shifts in rotavirus epidemiology before, during, and after the pandemic by analyzing retrospective data from the NRC in depth.

Materials and methods

Sample and data collection

Samples were collected within the framework of the NRC network for *Rotavirus A*. Within the NRC, every hospital or general practitioner in Belgium uses their own established methods (i.e., rapid antigen test, ELISA, qPCR panel test) to determine rotavirus positivity of stool samples. Clinical hospital laboratories and diagnostic centers can voluntarily send their rotavirus-positive stool samples to the university hospital (UZ/KU Leuven) for genotype characterization in the context of national surveillance purposes. For every shipped sample, related minimal patient data (e.g., date of birth, vaccination status, post code, date of sample collection) were forwarded to the NRC. All samples received by the NRC were subjected to genotyping (see *laboratory methods*).

Shape files of Belgium

The Shapefile of Belgium and its administrative units (version: 01.01.2023) were retrieved from the FPS Finance - General Administration of Patrimonial Documentation, Geoportal of Belgian Federal Institutions (28).

Laboratory Methods

Rotavirus Genotyping of VP7 and VP4

The stool samples were diluted to a 10% concentration in phosphate-buffered saline. Viral RNA extraction was performed using the QIAamp Viral RNA mini kit (Qiagen/Westburg, Leusden, The Netherlands) following the manufacturer's instructions. The extracted RNA was denatured at 95°C for 2 minutes. Reverse transcriptase PCR (RT-PCR) was conducted using the Qiagen OneStep RT-PCR Kit (Qiagen/Westburg) with previously described primers Beg9 and End9 (VP7), and VP4 1-17F and Con2Deg (VP4)(29). RT-PCR, PCR product identification, purification and sequencing were performed as previously described (5). Chromatogram sequencing files were quality controlled and analyzed using Chromas 2.5 (Technelysium, Helensvale, Australia). Samples were genotyped using the National Center for Biotechnology Information (NCBI, National Institutes of Health, Bethesda, MD) BLAST (Basic Local Alignment Search Tool) server on GenBank database.

Equine-like VP7 determination of G3P[8] sequences

All generated VP7 sequences of G3P[8] strains above 500bp were retrieved from the NRC database. Subsequently, we performed a BLASTn search of all G3P[8] VP7 sequences against predefined reference sequences representative of typical human and equine-like G3 rotaviruses (Equine-like VP7: MW280957.1, typical human VP7: MF469162.1), and used the closest hit to distinguish both G3 variants.

Statistical analyses

For the overall statistical analyses, all samples sent to the NRC as “rotavirus positive” were included, also those for which we could not confirm rotavirus using our genotyping PCR assays. The "Rotavirus season" was defined as spanning from August 1st to July 31st. Depending on the specific analyses, we excluded samples for which the relevant metadata were missing from both the descriptive figures and the statistical analysis. For age group statistics and seasonal distribution, this meant omitting samples with unknown dates of birth or collection, respectively. For spatial distribution figures, samples without a postcode were excluded, and for genotype figures and statistics, samples lacking genotyping results were not considered. To compile the vaccination data, individuals who received at least one dose of the vaccine were categorized as vaccinated.

To analyze differences between age groups across seasons and vaccination status compared to genotypes, a z-test for proportions was performed. Specific “outlier seasons” that were compared for each age group (2016-2017 and 2017-2018 for the 60+ age group- referred as “elderly”, 2021-2022 for the 2–5-year age group, respectively) were not included in the expected count estimations for the test of age groups. P-values obtained were adjusted using the Bonferroni correction method, with $p<0.01$ being deemed statistically significant. All analyses were conducted using RStudio (version 4.3.0) and all codes to perform the analyses and create figures are available at https://github.com/Matthijnssenslab/NRC_Belgium24.

Results

In total, 8024 samples were collected over the course of 14 rotavirus seasons, between July 2009 and August 2023. The NRC collected 5844 samples (72.8%) from Flanders (northern

Belgium, ~58% of the population), 877 samples (10.9%) from Wallonia (southern Belgium, ~31% of the population), and 120 samples (1.4%) from the Brussels Capital Region, with 1183 (14.7%) samples lacking postal code information (**Figure 1.A**). Before the pandemic, a mean of 622 samples (min:max, 298:991) were received per season, whereas this number was only 114 and 111 respectively during the two pandemic seasons (2019-2020 and 2020-2021). However, in the first season after the pandemic (2021-2022), a record of 1045 samples were received (**Figure 2.B**), whereas during the 2022-2023 season, the situation returned to pre-pandemic levels with 530 samples (**Supplementary file 1** for the geographical distribution of samples by year). Vaccination status information was available for 63.4% (5,111) of the 8,024 samples analyzed. Among these, 64.5% (3,300/5,111) were from patients vaccinated with Rotarix, 6.3% (322/5,111) were from individuals vaccinated with RotaTeq, and 29.2% (1493/5111) were from unvaccinated infants. Four infants were reported as vaccinated with both RotaTeq and Rotarix.

Rotavirus seasonality was disturbed during the pandemic, followed by a strong rebound

Before the pandemic, the epidemic peak of each season was observed in March or April, representing 20-40% of the cases per year (**Supplementary information 2**). However, during the pandemic this seasonal pattern was absent (**Figure 2**). As of October 2021, many pandemic measures were lifted, and schools restarted with full occupancy. Subsequently, an early and elongated peak was observed starting in December 2021. Interestingly, the peak of the second season after the pandemic (2022-2023) was again observed between March and April, similar to that of the pre-pandemic season, although it had a small tail until June.

High prevalence of 2-5-year-old rotavirus cases after the pandemic

During the 14 rotavirus seasons, an average of 70.1% (min:max, 54.3%:80.7%) of the cases were infants between the ages of 0 and 2 years (**Figure 3.A**), followed by children aged 2 to 5 years with an average of 20.3% of the cases (12.0%:33.2%). Only 4.8% (3.6%:7.2%) and 3.7% (0.33%:22.3%) of the cases belonged to the 5-18-year-old children and the elderly, respectively. For the elderly group, the 2016-2017 and 2017-2018 seasons were statistically significant outliers with 8.4% and 22.3% of the cases, respectively (**Figure 3.A,B**). Upon further inspection, we found that almost all the samples were sent to the NRC by the same hospital and were derived from a retirement home close to Mechelen (**Supplementary information**

3). A second observation was that during the pandemic (2019-2020 and 2020-2021), the age distribution of rotavirus cases was similar to previous years. However, in the 2021-22 season, after the COVID-19 pandemic measures were relaxed, the group of children aged between two and 5 years was significantly overrepresented compared to the average of the preceding 12 seasons ($p<0.001$, **Figure 3.C**).

G3P[8], G2P[4] and G9P[8] were the most common genotypes from 2009 to 2023

Overall, for 6352 out of 8024 samples (79.1%), genotyping of either VP7 or VP4 was successfully performed. During 14 seasons, the four most common genotypes were G3P[8] (n=2190; 34.4%), G2P[4] (n=1778; 27.9%), G9P[8] (n=944; 14.8%) and G1P[8] (n=496; 7.8%). Whereas the G1P[8] genotype was still responsible for 7.5-23.6% of the cases between 2009 and 2014, its prevalence decreased below 5% in eight out of the nine subsequent (2015-2023) seasons (**Figure 4.A**). Despite strong fluctuations, the G2P[4] genotype was the most common genotype between 2009-2021. The G9P[8] genotype prevalence steadily increased from 2009, reaching peak dominance in 2015-2016 (63.8%), after which it gradually decreased again in subsequent years, although a notable temporary emergence of the G9P[4] genotype occurred in 2018-2020 (**Supplementary Information 4**).

Remarkable dominance of Equine-like G3 strains in post-pandemic era of Belgium

Between 2009 and 2021, the G3P[8] genotype showed strong fluctuations, reaching a prevalence of over 50% in the 2018-2019 season. However, it completely dominated the two post pandemic seasons with 86.3% and 82.4% prevalence (**Figure 4.A**). Such dominance, and especially in two consecutive years, had not been observed before in Belgium. Given that in recent years, the G3P[8] genotype is known to circulate with either a Wa-like genotype constellation (typical human G3) or a DS-1-like genotype constellation (often referred to as equine-like G3), we further analyzed the VP7 sequences of all our G3P[8] strains, revealing a steady increase in the dominance of this equine-like G3 genotype over the typical G3 strain as of 2014 (**Figure 4.B**). In absolute numbers, these equine-like G3P[8] strains caused 561 and 271 cases in the 2021-2022 and 2022-2023 seasons, respectively. For the 2021-2022 season, this was the highest number of cases reported for a single genotype during our entire study period (**Supplementary Information 4**).

Increasing dominance of DS-1-like rotavirus strain over Wa-like strains

The predominance of G3P[8] strains with a presumed DS-1-like genotype constellation prompted a literature review of the most likely genotype constellation associated with various rotavirus G/P-genotype combinations. This search indicated that G1P[8], typical human G3P[8], G4P[8], G9P[8] and G12P[8] strains are usually found with a Wa-like genotype constellation, whereas G2P[4], the equine-like G3P[8] and G9P[4] strains are usually found with a DS-1-like genotype constellation (15–17,21,22). When coupling this information with our genotype prevalence data from our 14-years study period, we observed that the Wa-like strains dominated five out of the first seven years (2009-2016) of our surveillance, whereas DS-1-like strains dominated six out of the seven seasons (2016-2023) at the end of our study period (**Figure 4.C**).

Samples genotyped as equine-like G3P[8] revealed a significantly higher proportion of vaccinated infants

Over the course of the 14 seasons of rotavirus NRC activities, we observed a steady increase in the proportion of 0-to 2-year-old infants who received at least one Rotarix vaccine dose, while the overall estimated vaccination coverage was stable between 2011 and 2022 (**Figure 5.A.**). Next, we assessed genotype prevalence (splitting typical human and equine-like G3 strains) across vaccinated and unvaccinated infants (**Figure 5.B.**) and compared this to what would be expected if the genotype prevalence was the same in both groups, which should be the case if the vaccine protects against all genotypes equally well. Not unexpectedly, the G1P[8] genotype was over-represented in the unvaccinated group ($p<0.001$, expected: 118, observed:72), as this genotype is homotypic with the Rotarix vaccine strain (**Figure 5.C.**). Surprisingly, the opposite was observed for the equine-like G3 strain, which was over-represented in vaccinated infants ($p<0.001$, expected: 318, observed: 387) (**Figure 5.C.**). To check if this finding could be an artifact of the pandemic and/or the dominance of G3P[8] after the pandemic, we performed the same analysis only for the season 2021-2022. Interestingly, we observed the same phenomenon: samples from vaccinated infants encompassed 84.2% (197/234) of the equine-like VP7 G3P[8] and 67.7% (44/65) of the typical human VP7 G3P[8] genotyped samples (**Figure 5.D**).

Discussion

Global studies have highlighted a marked decline in communicable diseases like RSV, Influenza, strep and others during the COVID-19 pandemic, only to face a rebound later (30–32). Our research echoes these findings, showing a significant drop in rotavirus-positive samples sent for genotyping during the pandemic, with a record surge post-pandemic, the highest since our National Reference Center (NRC) was initiated. Under normal circumstances, rotaviruses are believed to be present ubiquitous in the environment and the human population, and infants are believed to be frequently re-exposed to wild-type rotavirus infections (or vaccination), recurrently boosting their immunity (33). We suggest that relaxed measures and lack of immune boost among children during the lockdown period led to a stark increase in rotavirus spread and subsequent clinical manifestations once the pandemic restrictions were lifted.

Historically, the rotavirus peak incidence in Belgium fell between December and February before vaccine introduction. As of 2006, after vaccine introduction, this peak shifted towards March and April (5,34). Our data from 2009-2019 supports this post-vaccination seasonal trend. However, pandemic measures nearly flatlined this pattern between March 2020 until October 2021 (**Figure 3**), when a strong resurgence occurred right after relaxation of measures. The following season began earlier than usual (December), most likely due to the accumulation of susceptible infants, resulting in the observed strong and long rotavirus season. By the 2022-2023 season, the seasonal pattern seemed to “return to normal” albeit it slightly prolonged to June.

The age distribution of *Rotavirus A* cases around the world is rather consistent with mostly children younger than 5 years of age being affected, followed by the elderly population. In our analyses, this known distribution was repeatedly observed before the pandemic, with one exception. An unusually high number of samples from an elderly population was recorded in the seasons 2016-2017 and 2017-2018. After consultation with the local doctor, this turned out to be an outbreak in one particular retirement home, which was detected because of a temporary change in the testing strategy (combined norovirus/rotavirus testing). In general, gastroenteritis outbreaks in the elderly are not routinely tested for rotavirus in Belgium, as only testing for the 0-5 age group is reimbursed. This case suggests that rotavirus outbreaks in the elderly population might remain largely undiagnosed. On the other hand, during the

initial year following the pandemic, there was a notable increase in the predominance in the samples from children aged 2 to 5 years, suggesting that the above-mentioned reduced exposure to rotavirus during the pandemic, impacted rotavirus circulation in this age group more than in other age groups.

Rotarix was introduced in Belgium in 2006, and vaccination rates were close to 90% in subsequent years resulting in significant changes in the epidemiology of rotavirus in Belgium (5). Prior to the vaccine introduction, the homotypic G1P[8] was the predominant genotype worldwide as well as in Belgium whereas after the vaccination, a shift towards the G2P[4] genotype was observed, not just in Belgium but also in other countries where Rotarix is the main vaccine employed (5,10–14). Our pre-pandemic surveillance data confirmed the fluctuating but high prevalence of G2P[4] in Belgium (**Figure 4.A**).

Rotavirus vaccination has been estimated to reduce rotavirus fatalities from 528,000 in 2000 to 215,000 in 2013, highlighting the significant impact of extensive rotavirus vaccination (35). While genotype proportions tend to fluctuate over time and geographic regions, the monovalent rotavirus vaccine Rotarix continues to be effective in preventing severe disease and hospitalizations, despite its slightly lower effectiveness against G2P[4] (24). Interestingly, we observed a gradual increase in the number of vaccinated patients' samples sent to the NRC, while vaccination coverage was stable in the same age group, coinciding with the increase in the number of equine-like G3P[8] strains. Our in-depth analysis shows that the sample proportion of vaccinated patients was higher in equine-like VP7 containing G3P[8] strains, which suggests that Rotarix might be slightly less effective against equine-like G3P[8] strains, compared to other genotypes, which might (partially) explain the high G3P[8] dominance in Belgium post-pandemic.

This equine-like VP7 G3P[8] genotype has been strongly associated with the DS-1-like genotype constellation (21,22,36). This specific constellation has also been prominently observed in conjunction with various other genotypes, including G9P[4] and G2P[4] (15–17). Moreover, immune response to rotavirus does not only target VP4 and VP7 but also have been shown to target the VP2, VP6, NSP2 and NSP4 proteins (37). Extrapolation of the rotavirus genotype constellation based on the available G/P genotype sequences (*see results),*

identified a strong dominance of DS-1-like strains in last 7 seasons (**Figure 4.C.**). This finding seems to confirm a prediction we made more than 10 years ago, that a relative enrichment of DS-1-like RVA strains (replacing the Wa-like constellation present in the Rotarix vaccine), might occur over time due to vaccine pressure (38). Recently, Degiuseppe *et al*. (2020) also reported that in four of the seven countries from Latin America, there was a rapid switch from Wa-like constellation to DS-1-like constellation, despite distinct vaccine introduction trends in each of the countries (12). Moreover, an increase in the proportion of samples from vaccinated infants over time and a higher incidence of vaccinated infants among cases infected with equine-like G3P[8] strains, align with possible positive selection of DS-1-like strains. It could be argued that this phenomenon cannot be explained only by DS-1-like genotype constellation, as we did not observe an over-representation of DS-1-like G2P[4] strains in the vaccinated infants (**Figure 4.C**). However, it could be counter-argued that the prolonged circulation of G2P[4] strains in the Belgian population after vaccine introduction (**Figure 4.A**) might have resulted in a good population immunity against the G2 VP7, which is not (yet) present against the equine-like G3 VP7. Overall, we speculate that the current dominance of the equine-like G3 strains in Belgium is a result of vaccine pressure towards DS-1-like strains, random stochastic variations and a bottleneck event cause by the pandemic.

Overall , we recommend that surveillance programs extend their focus beyond the VP7 and VP4 genotypes, towards other rotavirus gene segments. Such expansion could provide crucial insights into the impact of vaccination on the evolution of rotavirus genotypes and contribute to the development of next-generation vaccines that consider the full range of protective antigens.

In our study, several limitations should be acknowledged: 1) incompleteness of clinical data (e.g., vaccination data), as this information is provided voluntarily, and not always known by the laboratories sending us the samples; 2) possible selection bias due to the voluntary nature of sample submission to the NRC and more samples received from Flanders (North of Belgium); 3) we made assumptions about the Wa-like and DS-1-like genotype constellations of strains based on their G/P combinations, which was reported to be mostly correct, despite reports of occasional unusual genotype constellations (15–20). Nevertheless, our study

possesses significant strengths, notably its status as the largest and first nationwide assessment of rotavirus epidemiology in the context of a pandemic.

Conclusion

Our study on rotavirus epidemiology in Belgium post-pandemic indicates a significant increase in case numbers, particularly in 0-2- and 2-5-years old children, likely due to lack of exposure to rotavirus during the pandemic. The seasonality pattern has shifted during the pandemic, correlating with pandemic measures. Most notably, the G3P[8] genotype with equine-like VP7 and a DS-1-like genotype constellation has become the dominant strain for two consecutive years, co-circulating with typical VP7 containing G3P[8], replacing the most common G2P[4] genotype, which was the dominant strain for a decade. These findings highlight the evolving nature of Rotavirus A, exploring the need for future research to explore how the virus adapts in response to changes in vaccine policies and public health emergencies.

Statements

Ethical statement

This work was framed within the role of the National Reference Center for Rotavirus UZ/KU Leuven (as defined by the Royal Decree of 09/02/2011).

Conflict of interest

Authors declare no competing interests.

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Figures

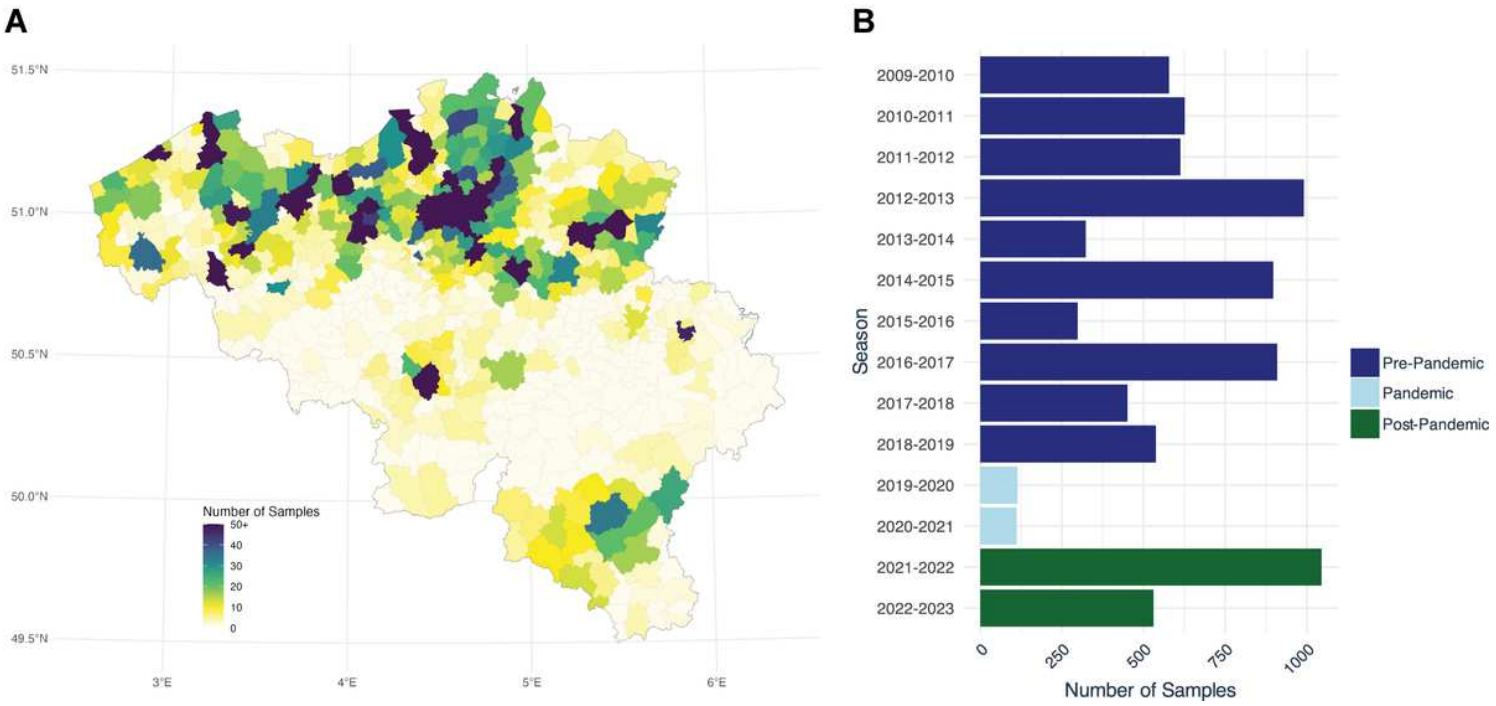

Figure 1

A. Overall spatial distribution of samples received by the NRC between the 2009-2010 and 2022-2023 seasons from each municipality of Belgium.**B.** Number of samples received by the NRC per season.

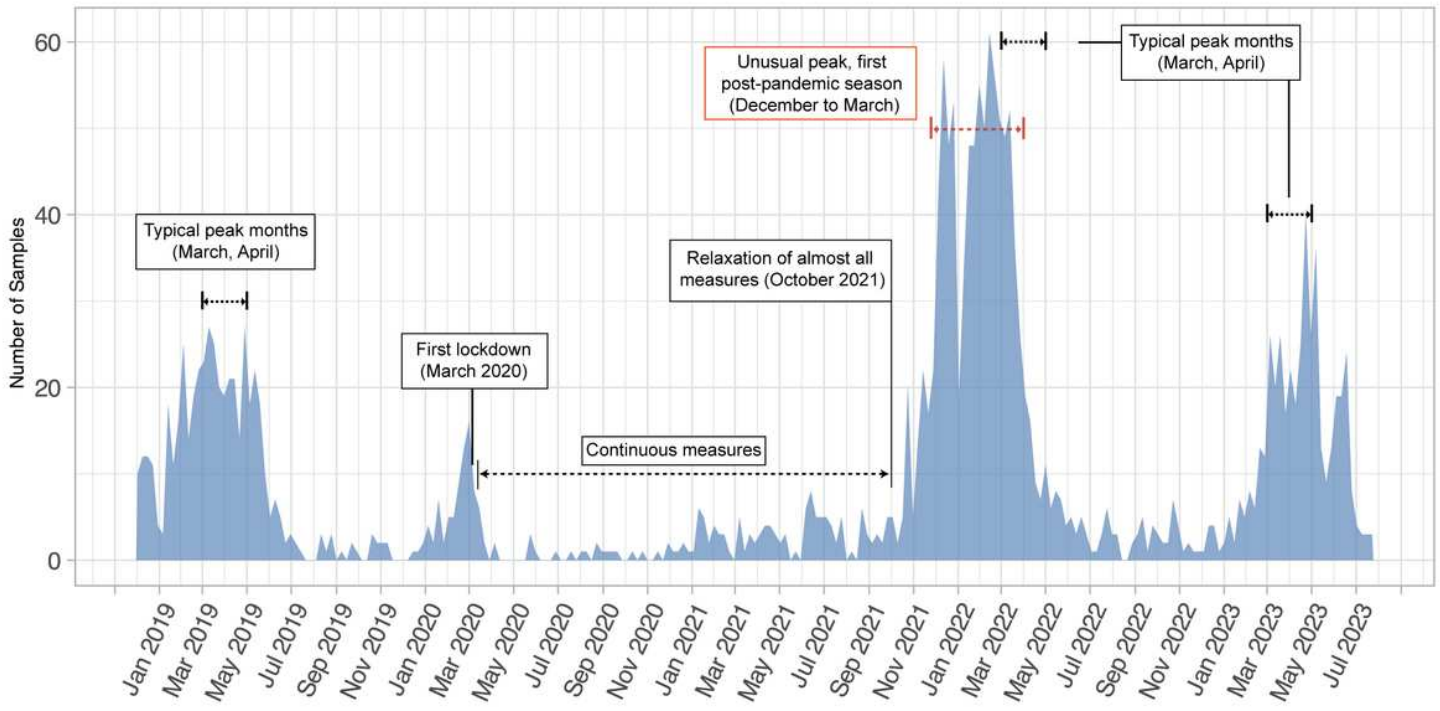

Figure 2

Weekly samples received by the NRC between December 2019 and July 2023.

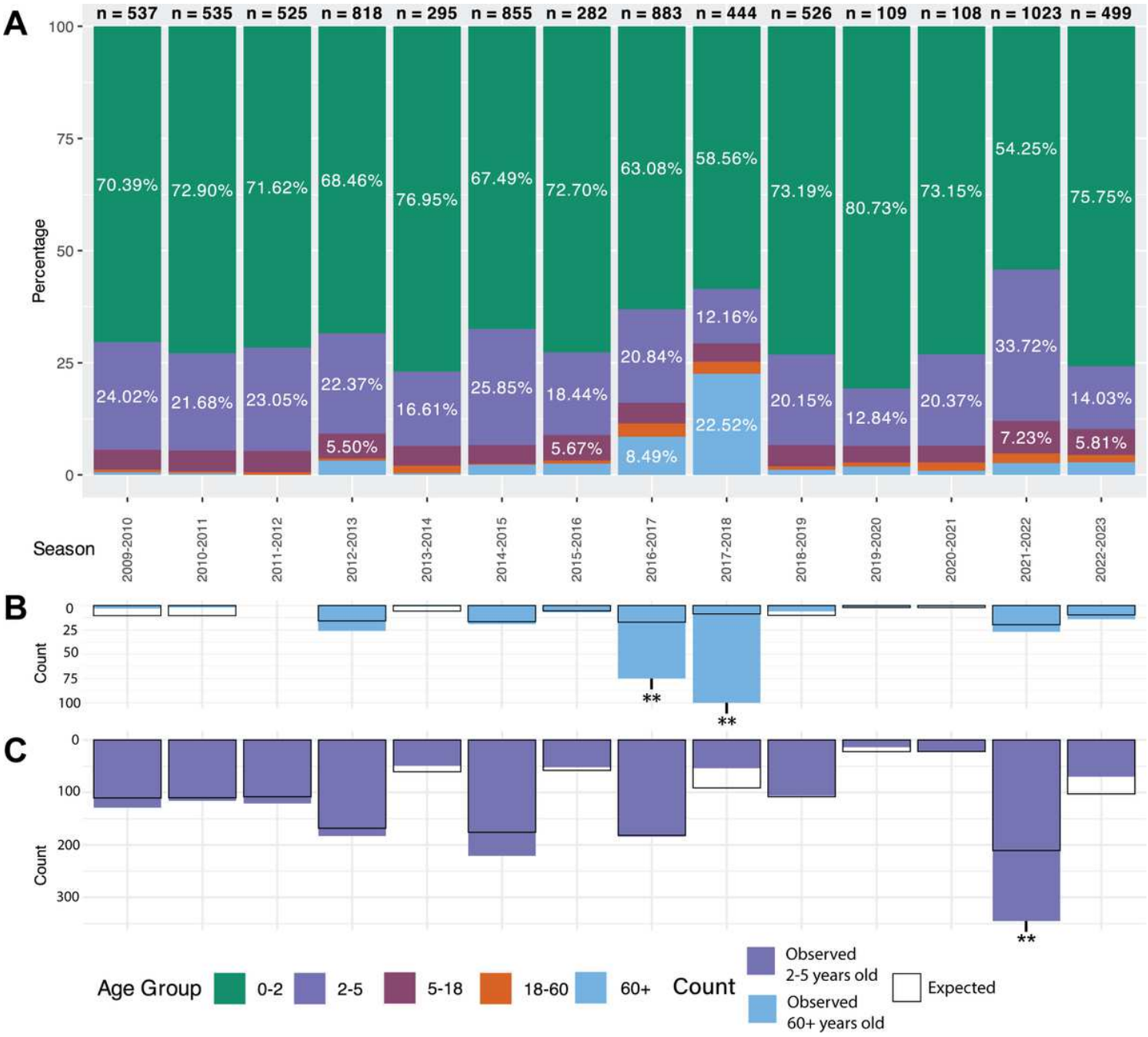

Figure 3

A. Age distribution of patients diagnosed with rotavirus over 14 consecutive seasons. **B.** Expected and observed sample counts of 60+ age group in different seasons. **C.** Expected and observed sample counts of 2-5 age group in different seasons. Tests: z-test for proportions, adjusted with Bonferroni correction (**, $p<0.001$).

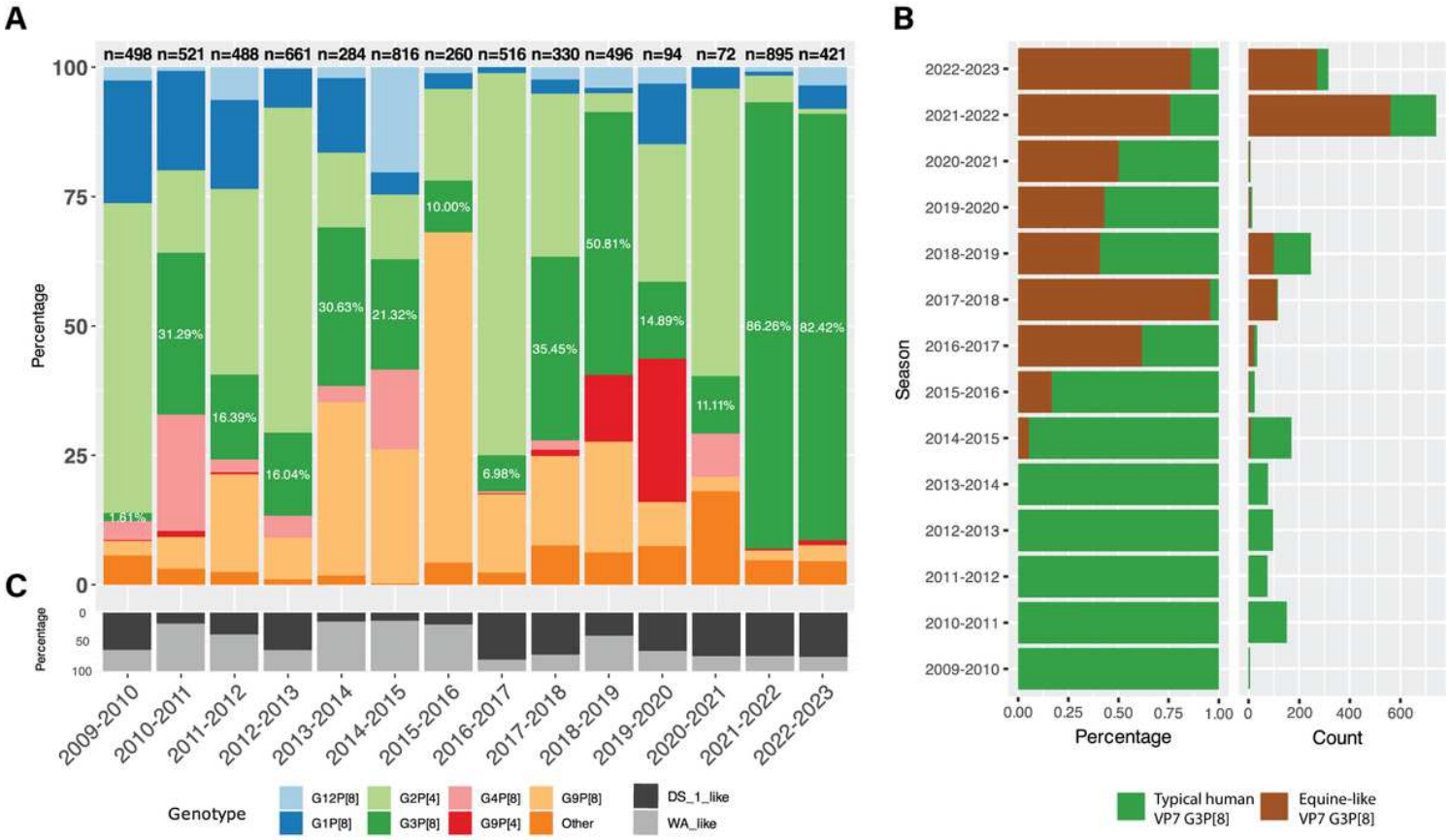

Figure 4

A. Genotype distribution across seasons. **B.** Relative and absolute numbers of typical and equine-like G3 VP7 strains across seasons. **C.** Inferred proportion of DS-1-like and Wa-like strains per season. For “Other” genotypes no assumption of the genotype constellations were attempted, and therefore they were excluded from Figure 4C.

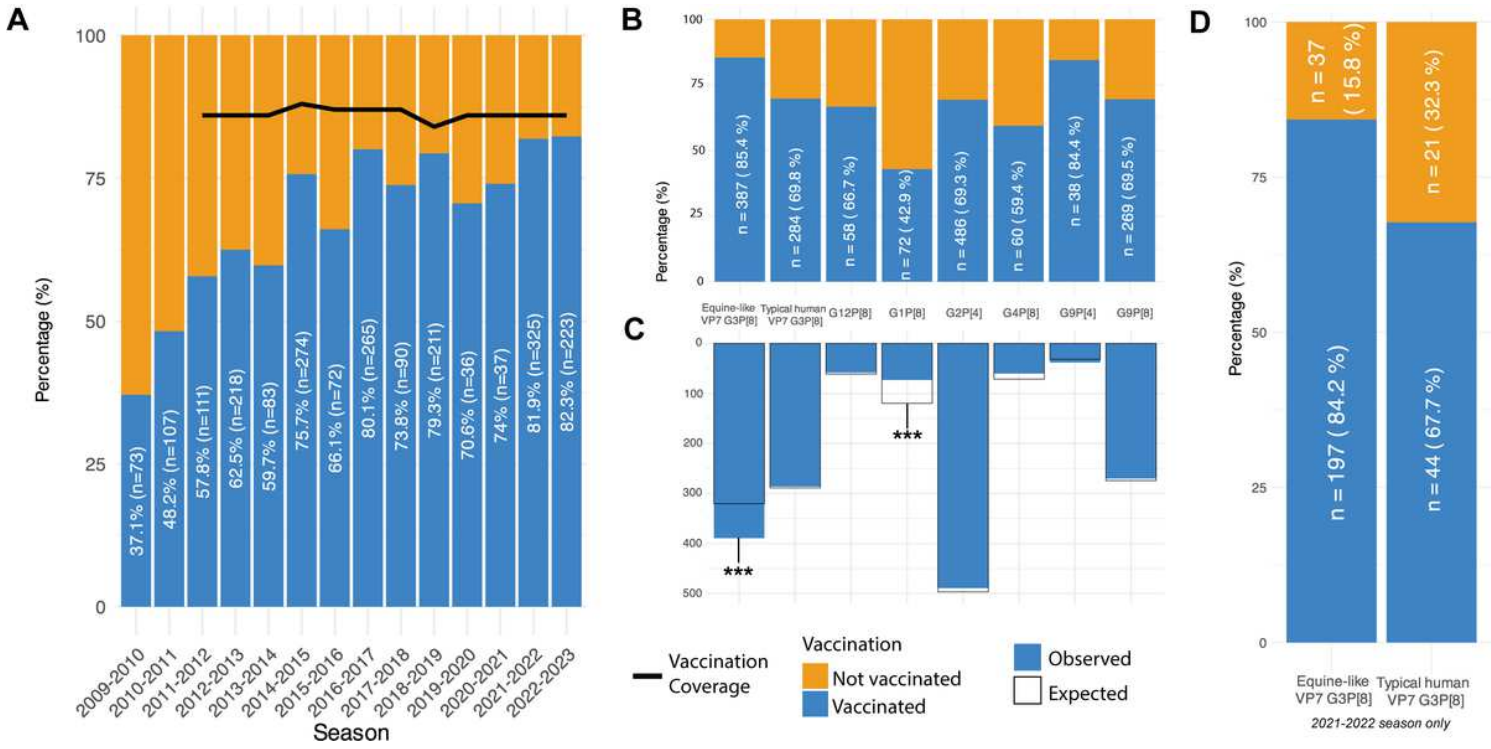

Figure 5

A. Proportions of vaccinated and unvaccinated patient samples of 0 to 2-years-old group per season. Vaccination coverage was retrieved from the data of WHO, Immunization coverage estimates by country (2022). **B.** Proportion of vaccinated vs. unvaccinated group of patients for each genotype. **C.** Observed and expected samples from vaccinated 0-2-years-old patients over the years per genotype, under the assumption that the vaccines protected equally well against all genotypes. Test: Z-test for proportions, adjusted using Bonferroni correction (**, p< 0.001). **D.** Proportion of vaccinated patient samples (only 0-2-years-old group) in the season of 2021-2022 for two distinct G3 subtypes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformation1.pdf
- Supplementaryinformation2.pdf
- Supplementaryinformation3.pdf
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