International Journal of Medical Microbiology 314 (2024) 151600

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Impact of the COVID-19 pandemic on norovirus circulation in Germany

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ARTICLE INFO

Keywords:
Norovirus surveillance
Dual typing
Molecular epidemiology
Germany
COVID-19 pandemic

ABSTRACT

Human norovirus is a major cause of viral gastroenteritis in all age groups. The virus is constantly and rapidly changing, allowing mutations and recombination events to create great diversity of circulating viruses. With the start of the COVID-19 pandemic in 2020, a wide range of public health measures were introduced worldwide to control human-to-human transmission of SARS-CoV-2. In Germany, control measures such as distance rules, contact restrictions, personal protection equipment as well as intensive hand hygiene were introduced. To better understand the effect of the measures to control the COVID-19 pandemic on incidence and the molecular epidemiological dynamics of norovirus outbreaks in Germany, we analyzed national notification data between July 2017 and December 2022 and characterized norovirus sequences circulating between January 2018 and December 2022. Compared to a reference period before the pandemic, the incidence of notified norovirus gastroenteritis decreased by 89.7% to 9.6 per 100,000 during the 2020/2021 norovirus season, corresponding to an incidence rate ratio (IRR) of 0.10. Samples from 539 outbreaks were genotyped in two regions of the viral genome from pre-pandemic (January 2018 to February 2020) and samples from 208 outbreaks during pandemic time period (March 2020 to December 2022). As expected, norovirus outbreaks were mainly found in child care facilities and nursing homes. In total, 36 genotypes were detected in the study period. A high proportion of recombinant strains (86%) was found in patients, the proportion of detected recombinant viruses did not vary between the pre-pandemic and pandemic phase. The proportion of the predominant recombinant strain GII.4 Sydney[P16] was unchanged before pandemic and during pandemic at 37.5%. The diversity of most common genotypes in nursing homes and child care facilities showed a different proportion of genotypes causing outbreaks. In nursing homes as well as in child care facilities GII.4 Sydney[P16] was predominant during the whole study period. Compared to the nursing homes, a greater variety of genotypes at the expense of GII.4 Sydney[P16] was detected in child care facilities. Furthermore, the overall proportion of recombinant strain GII.3[P12] increased during the pandemic, due to outbreaks in child care facilities. The COVID-19 pandemic had a high impact on the occurrence of sporadic cases and norovirus outbreaks in Germany, leading to a near suppression of the typical norovirus winter season following the start of the pandemic. The number of norovirus-associated outbreak samples sent to the Consultant Laboratory dropped by 63% during the pandemic. We could not identify a clear influence on circulating norovirus genotypes. The dominance of GII.4 Sydney recombinant strains was independent from the pandemic. Further studies are needed to follow up on the diversity of less predominant genotypes to see if the pandemic could have acted as a bottleneck to the spread of previously minoritized genotypes like GII.3[P12].

1. Introduction

Norovirus is a leading cause of acute gastroenteritis worldwide, associated with 18% diarrheal disease leading to 212,000 annual deaths worldwide (Lopman et al., 2016; Pires et al., 2015). People of all ages can be affected but young children and the elderly have the highest

Abbreviations: IRR, incidence rate ratio; MAFFT, multiple alignment using fast Fourier transform; NPI, non-pharmacological interventions; ORF, open reading frame; PBS, phosphate buffered solution; PCR, polymerase chain reaction; VP, viral protein.

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https://doi.org/10.1016/j.ijmm.2024.151600
Received 31 October 2023; Received in revised form 14 December 2023; Accepted 15 January 2024
Available online 18 January 2024
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incidence of disease and proportion of severe courses. Human noroviruses are very stable in the environment, they are transmitted via the fecal-oral route and can be spread directly from human to human via vomitus aerosols or indirectly via contaminated food or surfaces. In Germany, norovirus outbreaks are frequently associated with healthcare settings, community facilities, in particular child care facilities or nursing homes, predominantly in the winter months from November to April (Bernard et al., 2013).

Noroviruses are non-enveloped viruses, belonging to the family of *Caliciviridae* with a single stranded positive sense RNA genome. The genome contains three open reading frames (ORF). ORF1 encodes for the non-structural proteins, ORF2 for the capsid protein (VP1) and ORF3 for the minor capsid protein (VP2) (Robilotti et al., 2015). Noroviruses are classified into ten genogroups, with human noroviruses belonging to the genogroups GI, GII, GIV, GVII and GIX (Chhabra et al., 2019). They can be further divided into genotypes according to their ORF1 or ORF2 sequences. This dual typing is useful to characterize recombinants, as the usual recombination point in noroviruses is located in the ORF1/ORF2 junction region. According to the ORF1 nucleotide sequence, 60 polymerase (P) genotypes, and according to the ORF2 nucleotide sequences 48 genotypes could be distinguished (Chhabra et al., 2019).

Following the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in early 2020 and the evolving pandemic, extensive control measures were introduced in Germany, i.e. contact and travel restrictions, distance rules, personal protective equipment such as mandatory wearing of medical masks, cancellation of mass events, closures of day-care centers, schools, restaurants, hotels and shops, prohibition of meetings in clubs, sports and recreational facilities, compliance with hygiene rules in different facilities, isolation measures, and reinforced infection control measures in medical and long-term care facilities. This affected not only the spread of SARS-CoV-2 but also the transmission of all other circulating viruses transmitted from human to human as well as the monitoring of these pathogens (Dapper et al., 2022).

To evaluate a possible impact of the COVID-19 pandemic on norovirus diversity in Germany, we retrospectively analyzed trends in the molecular epidemiology of norovirus disease during the course of the COVID-19 pandemic using human stool samples from outbreaks and national surveillance data.

2. Material and methods

2.1. Ethics statement

Surveillance data were collected and data of molecular characterization of noroviruses were analyzed on the basis of routine national infectious disease surveillance duties by local and state health departments and the Robert Koch Institute as laid out in the German Infection Protection Act. Thus, a review by an ethics committee was not required. In accordance with §13 of the German Infection Protection Act, laboratories are permitted to send patient samples to national reference centers and consultant laboratories for further analysis. Stool samples sent to the consultant laboratory for noroviruses were characterized by genotyping of two genome regions.

2.2. Surveillance data

Symptomatic norovirus infections with laboratory confirmation have been notifiable in Germany since 2001. The detection of viral RNA by RT-PCR or detection of norovirus antigen is reported to the local public health department by the identifying laboratory. The health department completes and verifies case information according to the national surveillance case definition. Case data are anonymized and electronically transmitted to the state health department and, from there to the RKI, the national public health institute in Germany. We analyzed norovirus cases with laboratory confirmation notified between July 2017 and December 2022. We assessed time trends, comparing observed seasonal incidence during the COVID-19 pandemic to expected incidence. For this purpose, we defined a norovirus season as the time period between July of a given year and June of the following year (e.g. Norovirus season 2020/2021 refers to the cases of disease notified between July 2020 and June 2021). Expected incidence was calculated as the (stratum specific) mean incidence in a reference period before the COVID-19 pandemic (seasons 2017/2018 and 2018/2019).

We used Microsoft Excel and STATA 17 for data analysis and applied statistical tests as appropriate.

2.3. Samples

For genotyping of norovirus circulating in Germany before and during the COVID-19 pandemic we have analyzed stool samples from norovirus positive outbreaks of gastroenteritis and from sporadic cases with laboratory confirmation. An outbreak was defined as two norovirus cases with an epidemiological connection. Samples were sent to the Consultant Laboratory for Norovirus by local public health authorities, by diagnostic laboratories and by physicians.

2.4. PCR and sequence analysis

Samples were diluted in PBS (1:10) and spiked with an internal extraction- and PCR-control (MS-2 phage). Viral RNA was extracted from 140 µl suspension with QIAcube device using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) with an elution volume 60 µl. Determination of norovirus genotypes was done as previously described (Niendorf et al., 2020). PCR products were analyzed with Sanger sequencing and phylogenetic analysis were performed with Geneious Prime and MEGA 11.0.11 (Tamura et al., 2021). MAFFT algorithm was used for the alignment. In MEGA, best fit model of substitution pattern was determined and modeling of a Maximum Likelihood tree was done. The reliability of the branching pattern was tested with bootstrapping (1000 replicates).

3. Results

3.1. Time course of notified norovirus infections in Germany

Between July 2017 and December 2022, a total of 291,144 laboratory-confirmed cases of norovirus gastroenteritis were reported to the Robert Koch Institute. Fig. 1 depicts the monthly trend in case numbers. Notably, the incidence of norovirus sharply declined in Spring 2020, coinciding with the introduction of measures in Germany in response to the COVID-19 pandemic (Ullrich et al., 2021).

When compared to the mean annual incidence of 92.7 cases per 100,000 population during the reference period (norovirus seasons 2017/2018 and 2018/2019), the incidence dropped by 89.7% to 9.6 per 100,000 during the 2020/2021 norovirus season, corresponding to an incidence rate ratio (IRR) of 0.10. Twelve-month incidence also remained below expected values in the following season 2021/2022, although the reduction was not as pronounced (67.1 cases per 100,000; IRR=0.72) and evident only during winter 2021 and spring 2022 (see Fig. 2, panel A).

This pattern was generally present across several age groups (see Fig. 2, panel B through D). In young children, however, monthly incidence increased sharply in spring of 2021 and rose well beyond values of the reference period in the following summer and fall. The reduction of overall case numbers in the 2021/2022 season in young children compared to reference was also not as pronounced as in the other age groups (IRRs: 0.989 in 0 to 4 years old, 0.591 in 5 to 79 years old and 0.755 in 80 years and older people).

There was no statistically significant difference in monthly proportions of hospitalised patients between the pandemic seasons and the

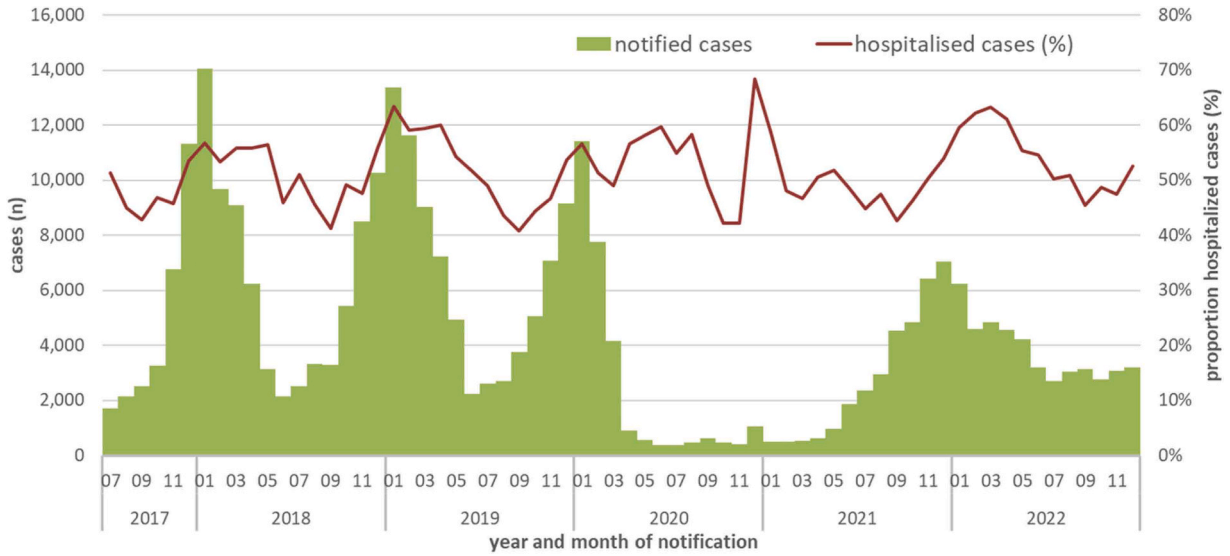

Fig. 1. Notified cases of laboratory-confirmed norovirus disease and proportion of hospitalised cases by month and year of notification, Germany, July 2017 through December 2022 (n = 291,144).

reference period (see Fig. 1, p = 0.86).

3.2. Description of norovirus outbreaks

In the period prior to the COVID-19 pandemic (January 2018 to February 2020), 2045 samples were sent to the Consultant Laboratory for Norovirus. Of those, 1444 samples (70.6%) could be assigned to a total of 639 outbreaks. Of these outbreak samples, a total of 539 (84.4%) could be genotyped completely in the ORF1 region as well as in ORF2 region, and only sequences of these samples were included in the genotyping analysis. The remaining 601 samples, according to the sample provider (mostly clinicians), were from sporadic cases with (no recognized) link to other cases.

During the COVID-19 pandemic phase (March 2020 to December 2022) in Germany, the number of norovirus-associated outbreak samples sent to the Consultant Laboratory decreased. In the period from March 2020 to December 2022, 755 samples were analyzed, of these 599 had an epidemiological link to 242 norovirus outbreaks. The remaining 156 samples were from sporadic cases without a known epidemiological link to a recognized outbreak. Samples from 208 (86%) outbreaks could be typed completely, and were included in this study. In about 90% of the received specimens belonging to outbreaks, information on the setting was provided. The largest proportion of samples from outbreaks were from child day care facilities, such as schools and kindergartens (41.9%; n = 369). Slightly more than a quarter of the analyzed samples from outbreaks came from nursing homes (27.8%; n = 245). Slight differences in the number of samples from outbreaks were detected for outbreaks in hospitals and in rehabilitation facilities. Fewer number of samples from outbreaks from both settings were sent to the Consultant Laboratory in the pandemic phase. Before the pandemic, 7.7% (n = 49) were hospital outbreaks; during the pandemic, this proportion dropped to 3.7% (n = 9). Before the pandemic, 2.8% (n = 18) were outbreaks in rehabilitation facilities, compared to 0.8% (n = 2) during the pandemic (see Fig. 3). A total of 4.4% (n = 39) outbreaks took place in other settings, like facilities for refugees or other special settings.

3.3. Diversity of norovirus genotypes in outbreaks before the pandemic

In the pre-pandemic phase a total of 539 outbreaks were genotyped by a single representative sequence, and 31 different genotypes were identified. Overall, recombinant viruses of genogroups GI or GII were detected in 86.4% (n = 466) of all outbreaks. Non-recombinant viruses of genogroup GII were detected in 4.3% (n = 23) and non-recombinant viruses of genogroup GI in 9.3% (n = 50) of the analyzed outbreaks. The most frequently detected genotype was GII.4 Sydney[P16], followed by GII.6[P7], GII.2[P16] with 37.5% (n = 202); 11.9% (n = 64) and 10.6% (n = 57), respectively (see Fig. 4).

3.4. Diversity of norovirus genotypes in outbreaks during pandemic

Compared to the pre-pandemic phase, the spectrum of detected genotypes was smaller during the COVID-19 pandemic, with only 25 different norovirus genotypes from 208 norovirus associated outbreaks. The proportion of GI, GII and recombinant viruses detected, 5.8% (n = 12) for GI, 9.1% (n = 19) for GII and 85.1% (n = 177) for recombinant viruses, is comparable to the pre-pandemic period. During the pandemic, changes were observed within the population of circulating noroviruses in Germany. The most frequent genotype was GII.4 Sydney [P16] with the same detection rate (37.5%) as before the pandemic, followed by GII.3[P12] whose proportion increased from 3.5% (n = 19) to 12.9% (n = 27). The detection rate of genotype GII.2[P16] slightly dropped from 10.4% (n = 56) to 7.2% (n = 15) in the pandemic phase. The proportion of the genotype GII.6[P7] also decreased from 11.9% (n = 64) to 1.9% (n = 4) during the pandemic (see Fig. 4).

3.5. Virus diversity in child care facilities compared to nursing homes

The majority of typed samples (69.9%; n = 522) from outbreaks were sent from child care facilities and nursing homes. Overall the diversity of norovirus genotypes associated with outbreaks was larger in child care facilities than in nursing homes. In child care facilities, 29 different genotypes were detected in 308 outbreaks, in nursing homes 20 genotypes in 217 outbreaks. In both facilities, genotype GII.4 Sydney [P16] was dominant, causing more than half of all outbreaks in nursing homes (58.9%; n = 126), but only about a fourth of the outbreaks in child care facilities (25.3%; n = 78). In child care facilities, the recombinants strain GII.2[P16] was detected in 13.3% (n = 41) of the outbreaks, in nursing homes in 4.2% (n = 9). A similar observation was made for the GII.6[P7] genotype, which was detected in 14.6% (n = 45) of outbreaks in child care facilities, compared with 1.9% (n = 4) of outbreaks in nursing homes. While the proportion of the dominant GII.4 Sydney[P16] genotype remained relatively constant over the period considered (23.7%, n = 52 pre-pandemic; 29.2%, n = 26 pandemic), a

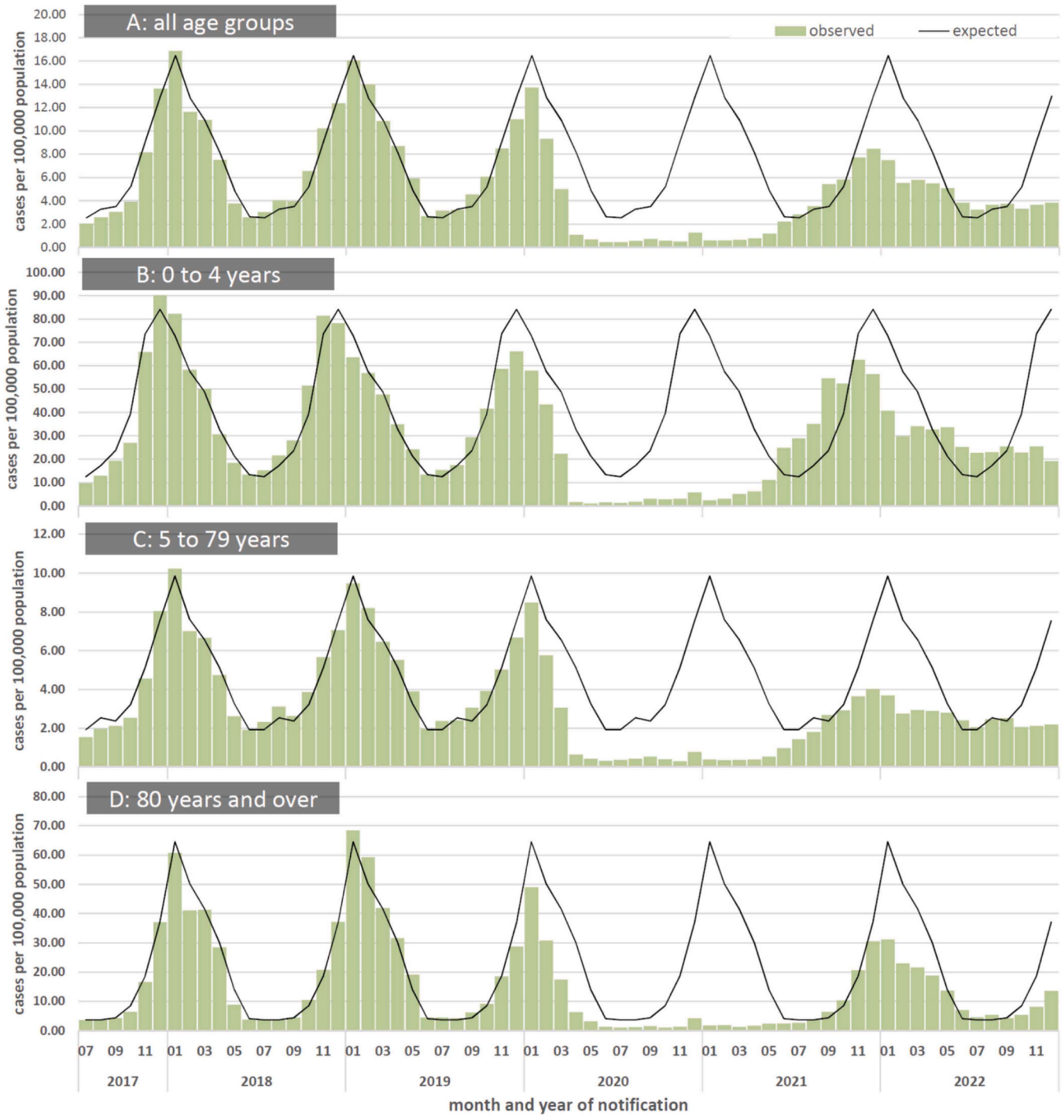

Fig. 2. Incidence of norovirus disease cases with laboratory confirmation by month and year of notification, observed (bars) vs. expected (line). Panel A shows overall incidence while panels B through D show incidence by age group (B: 0 to 4 years; C: 5 to 79 years; D: 80 years and over), Germany, July 2017-December 2022.

marked increase in the proportion of the GII.3[P12] genotype from 3.7% (n = 8) to 16.9% (n = 15) could be observed in outbreaks in child care facilities during the pandemic. The opposite effect was observed for the genotype GII.6[P7] in the child care facilities, whose distribution decreased from 19.2% (n = 42) to 3.4% (n = 3) during the pandemic (see Fig. 5).

The highly variable P2 region was used for phylogenetic analysis of sequences from the recombinant strains GII.3[P12] to highlight differences in the nucleotide composition in virus sequences before and during pandemic. For this analysis, 40 (n = 27 pre-pandemic and n = 13 pandemic phase) sequences from sporadic cases were additionally added to the 46 sequences obtained from norovirus associated outbreaks to increase quality of the phylogenetic analysis. The phylogenetic tree showed that a 73.9% (34 out of 46) of the pre-pandemic sequences clustered together (pre-pandemic cluster) and that 90% (36 out of 40) sequences of the pandemic samples clustered together (pandemic cluster) (see Fig. 6). There was a trend towards differential clustering of the sequences but the clusters were not completely divisible into pre-pandemic phase and pandemic phase.

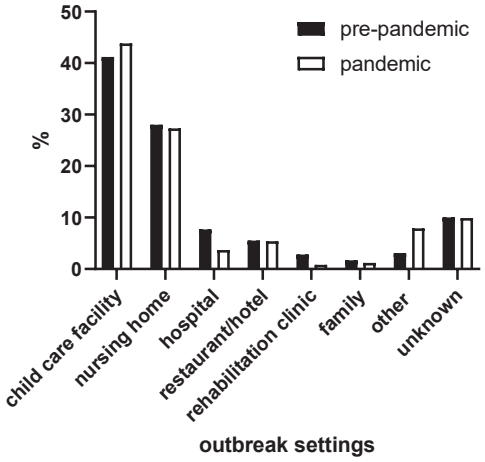

Fig. 3. Settings of analyzed outbreaks before and during the pandemic.

4. Discussion

The first infection of SARS-CoV-2 in a patient in Germany was diagnosed in January 2020 (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, 2020), until December 2022, six distinct SARS-CoV-2 waves were observed in Germany (Schilling et al., 2022). The first SARS-CoV-2 wave started in March 2020 and ended in May (week 20). First Measures to contain SARS-CoV-2 transmissions were introduced in March and were adapted over time (Bundesministerium für Gesundheit, 2023). Schools, day-care centers and shops closed in March 2020 and distance rules, contact and travel restrictions were introduced. In May 2020 first contact restrictions were relaxed. The second wave started in 2020 in September (week 40) and ended in 2021 week 8. In October 2020, restrictions on public life and social contacts were reinstated. The third SARS-CoV-2 wave was between January and June 2021 (week 9 to week 23). In April 2021 contact restrictions rules were introduced based on a seven-day incidence of 100. The fourth wave took place between August and December 2021; in November the epidemic situation of national importance ended based on the proportion of vaccinated and recovered persons. The fifth wave started in week 52 of 2021 and ended in week 21 of 2022; a sixth wave started in week 22.

In parallel with the implementation of various non-pharmacological measures aimed at curbing the distribution and impact of SARS-CoV-2 infections in spring 2020, a strong decrease in reported norovirus infections was observed in Germany, that was evident across age groups. During the norovirus season of 2020/2021, cases were suppressed to only 10% of the typical seasonal pre-pandemic numbers. In contrast, during the 2021/2022 season, the reduction was much less pronounced, and this reduction was observed primarily during the winter and spring months when also stricter COVID-related non-pharmacological interventions (NPIs) had been implemented. Norovirus infections are notoriously difficult to prevent and control, owing to their low infectious dose, high shedding titer, and environmental stability reviewed in (Barclay et al., 2014; Robilotti et al., 2015). This underlines the observation, that multiple strict measures have to be taken to successfully control norovirus outbreaks.

Notably, during summer and fall of 2022, there was a significant increase in monthly incidence of norovirus infections among young children compared to pre-pandemic levels, whereas other age groups did not experience such an increase. The underlying cause for this disparity between age groups remains unexplained. Nevertheless, it is possible that the higher prevalence of "static genotypes" circulating among children (Parra et al., 2017), in conjunction with the nearly complete suppression of infections in the previous season might have led to an increasing proportion of children susceptibility to these genotypes

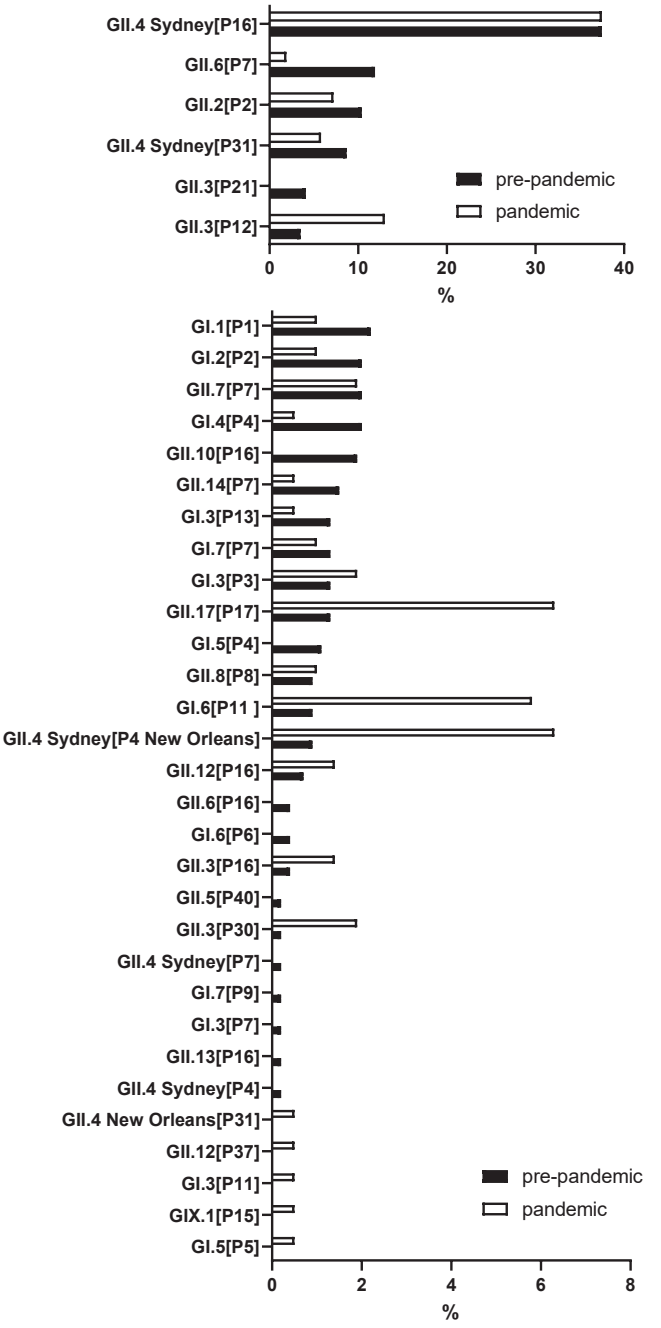

Fig. 4. Diversity of analyzed genotypes from outbreaks before and during the pandemic.

compared to before the pandemic. A similar effect has been observed with regard to RSV and other respiratory infections in children in Germany (Buchholz et al., 2023). It was shown by Kirsten Simmons and colleagues, that the basic reproductive number R_0 is highest in children under 5 years compared to other age groups (Simmons et al., 2013), likely due to higher rates of contact to both children and adults and lower levels of hygiene (Mossong et al., 2008). This could also explain the very rapid increase of norovirus infections in this age group, which became apparent after the COVID-19 measures were withdrawn.

While there was a significant alteration in healthcare-seeking behavior during the course of the pandemic, characterized by a reduction in visits to general practitioners and emergency departments (Boender et al., 2020), it is improbable that the observed decline in norovirus disease notifications can be attributed to this change. The

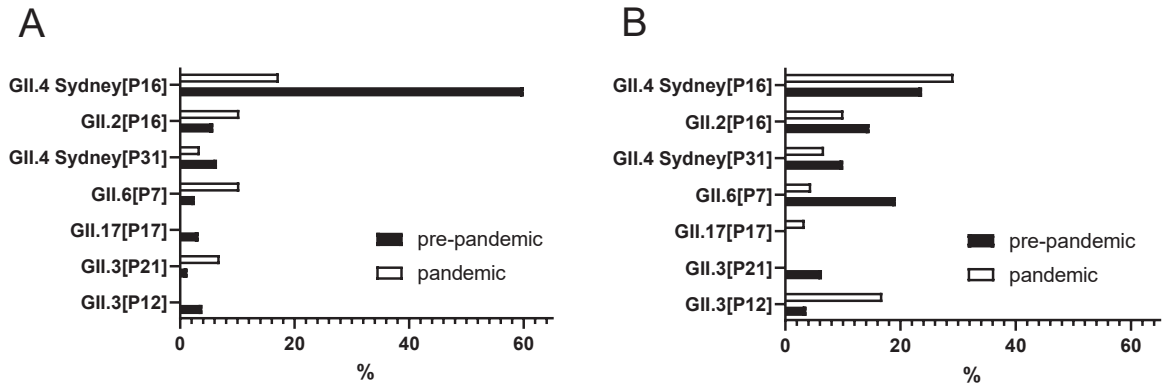

Fig. 5. Proportion of the seven genotypes most frequently detected in nursing homes (A) and child care facilities (B) before and during the pandemic.

stable proportion of hospitalized cases both prior to and throughout the pandemic implies that there was no discernible shift towards the exclusive diagnosis of severe cases of gastroenteritis. This observation suggests that the overall sensitivity of the reporting system also remained relatively stable. It is consistent with the steep decrease in positivity of norovirus antigen tests as observed in two large diagnostic facilities in Germany. They were found to be up to 20 times lower during compared to before the pandemic, and this difference was statistically significant (Eigner et al., 2021; Mack et al., 2021). Consequently, the decrease in norovirus cases is more plausibly attributable to the direct impact of NPIs on disease transmission within the population. Since the implemented NPIs primarily prevented direct person-to-person transmission and transmission through contaminated environments, the observed overall reduction in incidence during the 2020/2021 season may offer insights into the significance of these transmission routes over others (such as food- and waterborne) and corroborate findings from previous studies (Belliot et al., 2014).

Through the molecular surveillance of noroviruses in Germany, presented in this study, we described the epidemical characteristics of norovirus infections and the genetic diversity of circulating norovirus strains in Germany. The study was conducted over a period of five years from January 2018 to December 2022 and thus included a pre-pandemic phase from January 2018 to February 2020 and the pandemic phase between March 2020 and December 2022. During the study period a very high proportion of about 86% (n = 747) of recombinant noroviruses was detected in outbreak cases. This is in line with other studies worldwide, which detected a very high number of recombinant viruses (Cannon et al., 2021; Li et al., 2023; Navarro-Lleo et al., 2022; Phengma et al., 2023). The proportion of recombinant viruses did not differ between the pre-pandemic and pandemic phase in Germany. Due to the long period of our study of five years, a large number of norovirus associated outbreaks (n = 747) could be characterized. This very large number of characterized samples made it possible to detect the circulation of even very rare genotypes in Germany. By genotyping in both regions, we got a great depth of detail and could detect a high proportion of rare recombinant viruses in this study. This included rare recombinant viruses such as GI.3[P7], GII.3[P30], GII.12[P37] or GII.5[P40]. A total of 36 different norovirus genotypes were identified in Germany. In a global study reported by Jennifer Cannon and colleagues 1325 dual typed norovirus sequences submitted to NoroSurv between September 2016 and August 2020 from 16 countries were included. During this period, sequences from a total of 31 different norovirus genotypes were submitted to the database (Cannon et al., 2021).

The genotype GII.4 Sydney[P16] was predominant in Germany as well in the pre-pandemic and in the pandemic phase with the same detection rate of 37.5%. This is consistent with surveillance data from many countries around the world, where GII.P16-GII.4 Sydney was most frequently detected in the study period (Cannon et al., 2021; Navarro-Lleo et al., 2022; Phengma et al., 2023). In some countries, however, the GII.4 Sydney[P31] genotype was also reported to be dominant; in particular, studies from China, Hong Kong and Japan reported a dominance of this genotype (Cannon et al., 2021; Li et al., 2023; Pham et al., 2023). The difference of diversity of circulating noroviruses was shown by two studies from in Spain. Navarro-Lleó and colleagues typed noroviruses from sporadic infections in all age groups between January 2016 and April 2020. In this study, the most frequently detected genotype was GII.4 Sydney[P16] and GII.4 Sydney[P31] was the third most frequently detected genotype (Navarro-Lleo et al., 2022). In the second Spanish study, in which norovirus-associated outbreaks were typed in all age groups between 2017 and 2019, the most frequently detected genotype was GII.4 Sydney[P31] followed by GII.4 Sydney [P16] (Anfruns-Estrada et al., 2022). The results of these studies lead us to conclude, that genotype diversity must always be considered in the context of the sampling date and region. In addition, the diversity can be influenced by the age groups sampled, but also by whether sporadic cases or outbreaks are analyzed. Furthermore, the circulation of noroviruses could vary from region to region, which was also seen in our previous study in Germany (Niendorf et al., 2020). When comparing the diversity of circulating noroviruses in Germany before and during the pandemic, it was found that the proportion of some strains showed only very few changes during this period (GII.4 Sydney[P16], GII.2[P16] and GII.4 Sydney[P31]). Other strains were detected more frequently before the pandemic than during the pandemic (GII.6[P7] and GII.3[P21]). In contrast, the genotypes GII.3[P12], GII.17[P17] and GII.4 Sydney[P4 New Orleans] were detected more frequently during the pandemic than before the pandemic. So far, there are still very few studies comparing the diversity of circulating noroviruses during the pandemic with pre-pandemic data. Most of these studies focus on norovirus typing only in ORF2, which creates a bias in the distribution because it is not possible to distinguish between recombinants. Khamrin and colleagues described in a study the diversity of noroviruses in Thailand in 2017 and 2018 (pre-pandemic phase). They were able to show that the GII.2[P16] genotype, which was dominant until 2017, was displaced by the GII.4 Sydney[P16] and GII.4 Sydney[P31] genotypes (Khamrin et al., 2022). The distribution of genotypes also changed in Thailand with the COVID-19 pandemic. The genotype GII.3[P12] had not been detected in two studies previously published studies from Thailand, in which samples collected between 2005 and 2018 (pre-pandemic phase) were analyzed (Khamrin et al., 2022; Supadej et al., 2017). In the third study, samples were genotyped from January 2019 to December 2020 (Phengma et al., 2023), covering the beginning of the COVID-19 pandemic. It was shown that the GII.3[P12] genotype was the second most frequently detected after the dominant GII.4 Sydney[P16].

To identify trends in the diversity of norovirus in more detail, most common genotypes of outbreaks in nursing homes were compared to genotypes of outbreaks in child day care facilities in pre-pandemic and pandemic time period. Measures to prevent the spread of SARS-CoV-2 were less stringent in the child care facilities than in the nursing

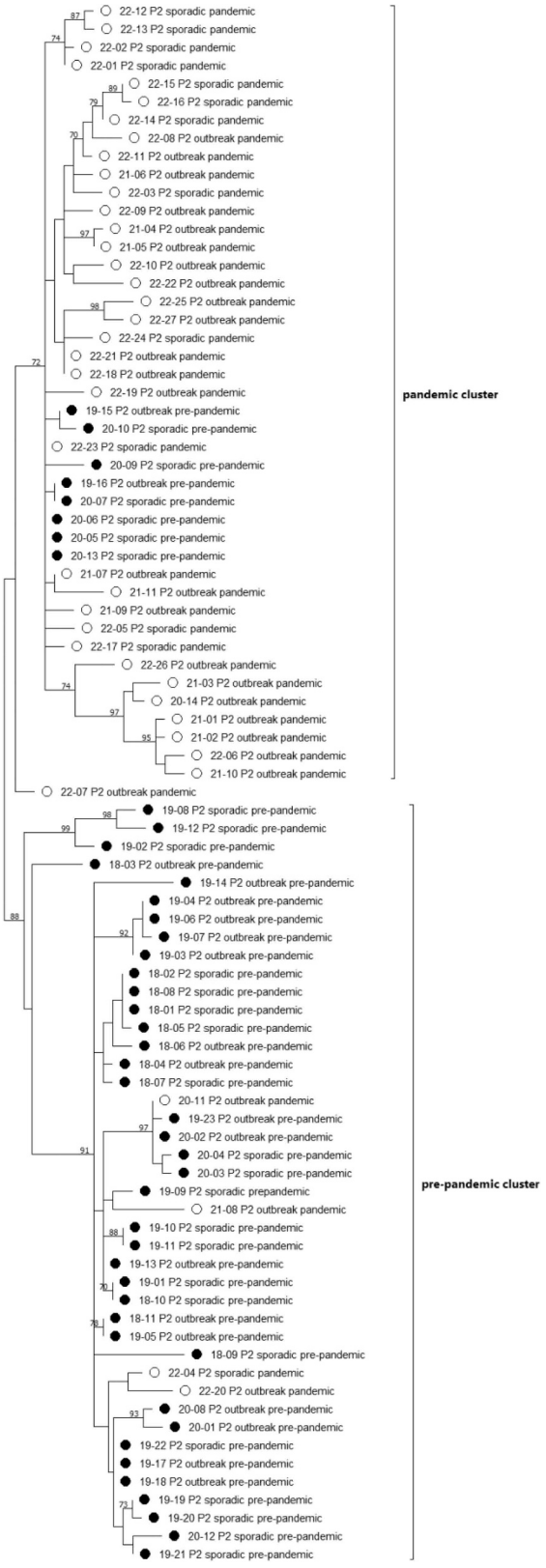

Fig. 6. Phylogenetic analysis of a 619 base pair fragment of P2 domain (ORF2) from norovirus genotype GII.3[P12] (sporadic and outbreaks) analyzed in this study. German sequences from the pre-pandemic phase were marked with a filled dot, sequences from samples during the pandemic were marked with an empty dot. The phylogenetic tree was reconstructed using Maximum Likelihood method with Bootstrap test (1000 replicates) and Kimura 2-parameter method available in MEGA 11.0.11. Bootstrap value above 70 were shown.

homes. Despite the lockdowns and general closures of child care facilities, emergency care was available throughout the pandemic for a proportion of children. In nursing homes, measures additional to increased hygiene were implemented, such as limitations to the number of guests, regular testing for staff, inhabitants and guests and isolation of infected persons. In nursing homes four genotypes were mainly identified in both time periods: GII.4 Sydney[P16], GII.4 Sydney[P31], GII.3 [P12] and GII.17[P17]. Interestingly three genotypes were circulating in low proportions in nursing homes before the pandemic: GII.2[P16], GII.6[P7] and GII.3[P21] but these recombinants could not be detected during the pandemic. In child day care facilities, the five most frequent genotypes were detected in both time periods. Genotypes GII.2[P16], GII.6[P7] were still detectable in children during pandemic in contrast to nursing homes, whereas GII.17[P17] was only present in children in the pre-pandemic phase. In a study conducted in Japan (2007–2015) with regard to norovirus genotype distribution in children and older people, more genotypes (7−13) were found in outbreaks in kindergarten/nursery schools and primary schools as in outbreaks in nursing homes (1–5 genotypes) (Kumazaki and Usuku, 2016). In 2007–2015 the dominant genotype was GII.4 especially in nursing homes in Japan, which is consistent with our results and other reports (Cornejo-Sanchez et al., 2023; Kumazaki and Usuku, 2016; Vega et al., 2014). The most common genotypes found in child day care facilities in Germany were also found in Japan (Kumazaki and Usuku, 2016). A further report revealed that norovirus genotype diversity was greater in community-acquired infections as in inpatients (Franck et al., 2015). Petrignani and colleagues did a meta-analysis for norovirus introduction routes into nursing homes and risk factors for the viral spread. They identified that a high staff-resident contact intensity is a risk factor for norovirus infection of residents and staff. Less mobile residents are less likely to spread the virus but the analysis was not able to evaluate whether the norovirus introduction was linked to symptomatic or asymptomatic persons (Petrignani et al., 2015). Parra and colleagues investigated the evolutionary pattern of the capsid protein from different norovirus genotypes and defined static and evolving genotypes (Parra et al., 2017). They put forward the hypothesis that older people are more likely to become ill by evolving genotypes like GII.4 because of their acquired immunity against static genotypes. In a study of Lindesmith and colleagues toward immune imprinting of genotype GII.4 they discuss, that GII.4 variants cocirculate and evolve especially in children. Adults already have a history of exposures to GII.4 variants and immunological back-boosting result in a mismatch between the actual circulating GII.4 variant and the corresponding immune response (Lindesmith et al., 2022). This could be another piece of the puzzle to explain the GII.4 dominance in the elderly in German nursing homes. It was also stated, that norovirus transmission in nursing homes is more restricted due to limited mobility and therefore promoted the increased GII.4 rate in a short period of time, which is also promoted by the age-related limitation of the immune system (Kumazaki and Usuku, 2023).

Genotype GII.3[P12] was detected in elderly people at a low rate but in higher proportions in children before and during the pandemic. The phylogenetic analysis of sequences encoding for a part of capsid region of GII.3[P12] of outbreaks showed, that there was a cluster of sequences before and separate cluster during pandemic. To get more information about this clustering, German GII.3[P12] sequences from sporadic cases before and during pandemic were added to this analysis. The trend of sub-clustering remained unchanged, which could be an indication that the genotype has continued to evolve during this time period into a subcluster. Whether SARS-CoV-2 control activities have influenced this clustering cannot be answered but monitoring of the evolution of this genotype would be still interesting, therefore further analyses of more sequences with complete VP1 sequences have to be done. The proportion of GII.3[P12] infections in China has increased since 2009–2012 (Fu et al., 2021; Lu et al., 2023) and this genotype was also found in Thailand in children (Phengma et al., 2023). In contrast to the high

proportion of 30% GII.3[P12] in Asia, this genotype was detected in Spain in only 1.4% and 0.4% of sporadic cases in 2019 and 2020, respectively (Navarro-Lleo et al., 2022). In the Chinese and Spanish studies, this genotype was associated with sporadic cases in children. Data from China showed high infection rates of GII.3[P12] in infants (<1 year) (Fu et al., 2021; Lu et al., 2023). In a different study, the humoral immune response to norovirus genotypes was investigated (Villabruna et al., 2022). It was shown that the seroprevalence against GII.3 was 70% in 0.6 months old children (probably due to maternal antibodies). In > 6–12 months old children this prevalence dropped sharply and then increased in older children (>1.5–5.5 years). An IgG response against GII.3 could only be found in > 1.5–5.5 years old children, suggesting that this genotype elicits a profound immune response that may be lower in the elderly due to their impaired immune status, leading to reinfection during outbreaks in nursing homes. It would be interesting to verify if the genotype is causing outbreaks or sproradic infections in the nursing homes worldwide.

5. Conclusions

Our study evaluates the impact of the COVID-19 pandemic on the incidence and diversity of circulating noroviruses in Germany. The presented data suggest that the NPIs had a significant effect on the incidence of norovirus infections in Germany. It could be shown that the incidence of notified norovirus infections decreased massively with the introduction of the NPIs. The reduction of these intervention measures in spring 2021 led to an increase in norovirus infections, which were first detected in children under 5 years of age. In contrast, the impact of the COVID-19 pandemic on the diversity of circulating noroviruses in Germany appears to have been relatively low. The detection rates of the dominant genotype GII.4 Sydney[P16] were identical before and during the pandemic. The circulating genotypes in patients, who are particularly sensitive to norovirus infections was comparable in elderly and children. The diversity of outbreaks was higher in child care facilities compared to nursing homes. In nursing homes, the rate of different circulating genotypes was decreased during the pandemic butGII.4 Sydney[P16] was still dominant, which was probably an effect of control activities against the transmission of SARS-CoV-2 like contact restriction, visit restrictions, restrictive hygiene measures. This bundle of activities leads to less human-to-human transmission of several viruses. The question of the described increase in the detection rate of genotype GII.3[P12], especially in outbreaks in child care facilities, could not yet be conclusively clarified in this study and requires further investigation.

Funding source

This research received no external funding.

CRediT authorship contribution statement

Niendorf Sandra: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Bock C.-Thomas:** Funding acquisition, Writing – review & editing. **Mas Marques Andreas:** Data curation, Formal analysis, Writing – review & editing. **Altmann Britta:** Data curation, Formal analysis, Writing – review & editing. **Faber Mirko:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jacobsen Sonja:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Acknowledgements

We thank Ute Obst, Kathrin Stanossek, Anja Lerch, Steffen Zander and Daniel Kießig for excellent technical assistance. We thank the staff of local public health authorities and all laboratories for provide samples and data.

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