

# NATIONAL REFERENCE CENTRE NOROVIRUS

Annual report 2021

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# WHO WE ARE

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Sciensano can count on more than 950 staff members who are committed to health every day.

As our name suggests, science and health are central to our mission. Sciensano's strength and uniqueness lie within the holistic and multidisciplinary approach to health. More particularly we focus on the close and indissoluble interconnection between human and animal health and their environment (the "One health" concept). By combining different research perspectives within this framework, Sciensano contributes in a unique way to everybody's health.

For this, Sciensano builds on the more than 100 years of scientific expertise.

## Sciensano

Infectious diseases in humans - Foodborne pathogens  
NRC Norovirus

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## **EXECUTIVE SUMMARY**

In 2021, the National Reference Centre (NRC) received reports of 32 outbreaks potentially linked to Norovirus, affecting a minimum of 241 individuals. Confirmation of Norovirus as the causative agent was established in 22 of these outbreaks, affecting a minimum of 215 individuals. Subsequent typing via sequencing allowed determination of Norovirus genogroup and genotype in 21 outbreaks, while technical limitations precluded such determination in the remaining case. Among the typed outbreaks, Norovirus genogroup GI was identified in 1 instance, while genogroup GII was predominant, detected in 20 outbreaks.

Of the reported outbreaks in 2021, 4 were suspected to involve Norovirus transmission through food. Norovirus was detected in human samples in all of these outbreaks. Specifically, in 2 outbreaks, the transmission via food could be confirmed. Notably, the majority of Norovirus reports originated from residential institution such as nursing home, prison, boarding schools and households.

In 2021, the predominant circulating strain of Norovirus remained GII.4 (Sydney 2012) with 6 outbreaks, initially identified in September 2012 by van Beek et al. Additionally, Norovirus genotype GII.17 was implicated in 2 outbreaks during this period.

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# OVERVIEW OF ACTIVITIES

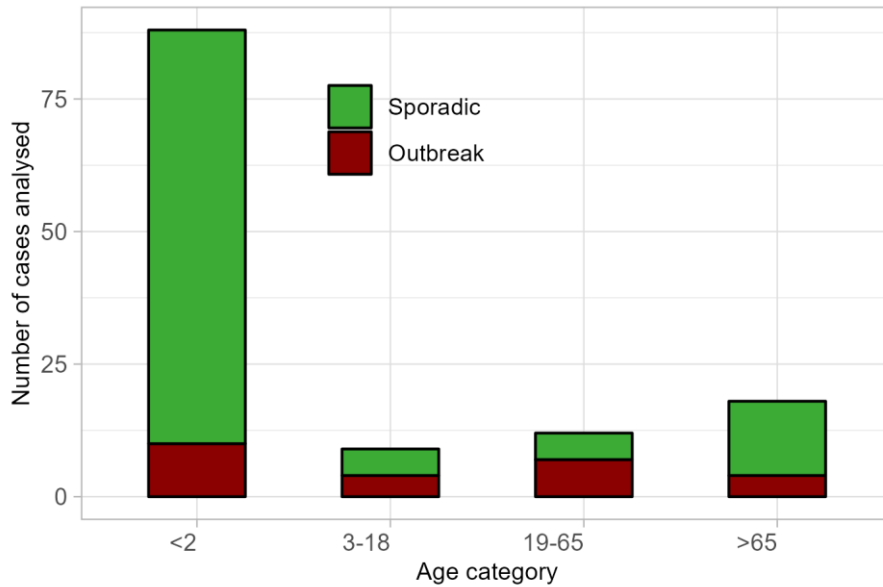
## 1. Norovirus detection

In 2021, the NRC Norovirus received a total of 218 human samples (see table 1). Diagnostic detection of human pathogenic Norovirus by reverse transcriptase (RT-)PCR was conducted on 206 of these samples, revealing the presence of the virus in 128 cases. Additionally, 12 samples were sent to the NRC after norovirus had already been detected at the clinical lab using an RT-qPCR method.

**Table 1.** Samples NRC norovirus 2021

	Samples received
Total	218
Norovirus detected	140
Norovirus not detected	78
Outbreak	36
Sporadic	104

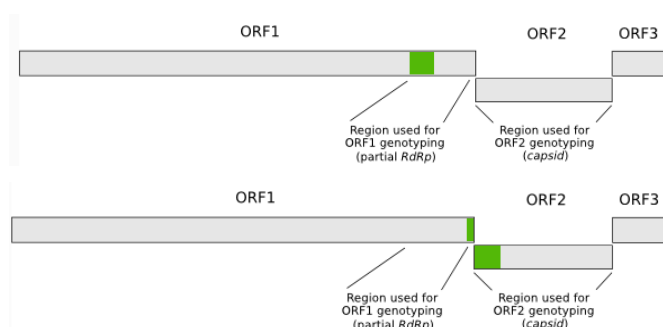
A total of 140 samples tested positive for norovirus. Among these, 36 were identified during an outbreak, while the remaining 104 were categorized as sporadic cases. Figure 1. illustrates the distribution of samples received by the NRC across different age categories. Norovirus was predominantly detected in the youngest age category (<2 years old). Notably in 2021 the NRC received a markedly reduced number of samples from the oldest age category (>65). Given that the majority of cases associated with outbreaks typically occur within this elderly demographic, this resulted in a reduction in the total number of reported outbreaks compared to the norm. It is worth noting that for some positive samples, birth dates were not provided, leading to their exclusion from the analysis (n=13).



**Figure 1.** Norovirus detected in 2021 per age category.

## 2. Norovirus genotyping

The aim of variant determination is to further molecularly characterise positive norovirus samples by typing via sequencing. In this way, the spread and evolution of norovirus can be mapped. For this purpose, two differentiating regions of the NoV genome were sequenced. The genome of norovirus is encoded by 3 open reading frames: ORF1 (polymerase), ORF2 (major capsid, VP1) and ORF3 (minor capsid, VP2) (figure 2). The genotypic and variant classification is made possible by the sequencing and bioinformatic homology analysis of different regions in the polymerase or in the major capsid protein. Both regions are located at the boundaries of ORF1 and ORF2 respectively and represent the hotspot for recombination within the norovirus genome. In 2019 the international norovirus classification-working group provided an update to the current classification scheme for norovirus to cover the new unassigned virus types (Chhabra *et al.*, 2021).



**Figure 2.** Schematic representation of the location of genomic regions used for genotyping of Norovirus (Vinjé *et al* 2004).



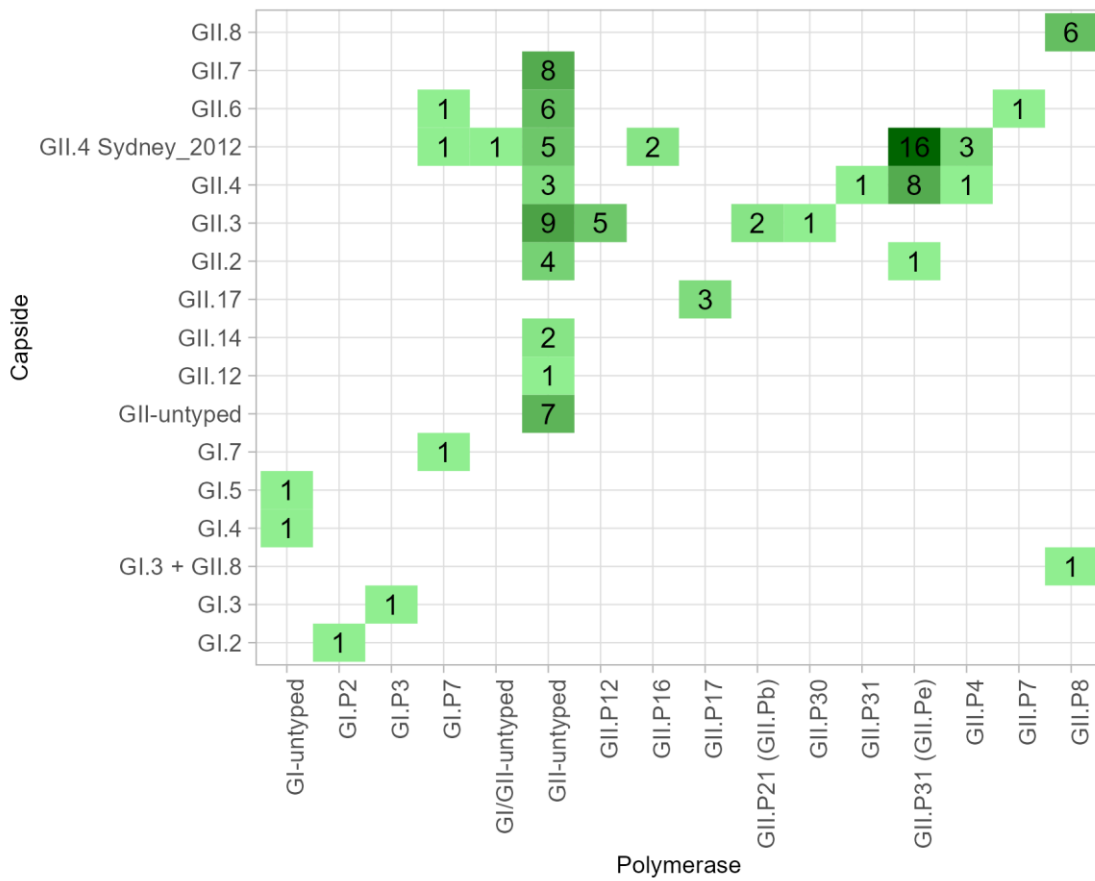
A total of 130 samples underwent genotyping, with the NRC successfully sequencing either the capsid region, the polymerase region, or both for 123 samples. Some genotypings were not achieved possibly due to mutations at the level of the primers used for amplification of the capsid region. For these cases the NRC confirmed the presence of norovirus.

Among the 130 samples, 31 samples were tested within the framework of a reported outbreak, with a maximum of five samples per outbreak being typed. An initial serogrouping assay showed that 1 of the samples contained the human pathogenic genogroup GI, 28 the human pathogenic genogroup GII and none both. The remaining 99 samples originated from sporadic cases. The serogrouping assay showed that 4 of the samples contained the human pathogenic genogroup GI, 82 the human pathogenic genogroup GII and 4 both.

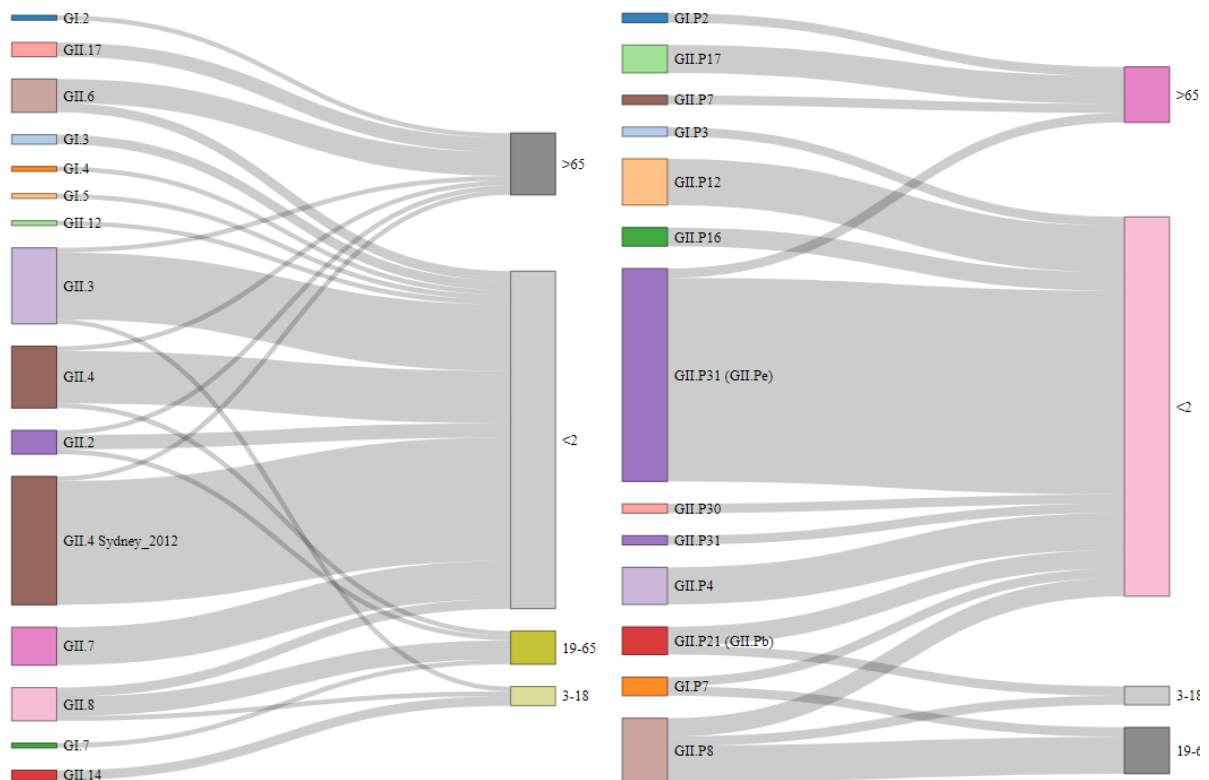
Based on polymorphisms detected in the capsid gene, 5 different genotypes within genogroup GI (GI.2; GI.3; GI.4; GI.5; GI.7) are distinguished in Belgium in 2021. A total of 10 different genotypes distinguished for genogroup GII (GII.4 Sydney 2012; GII.3; GII.4; GII.6; GII.7; GII.8; GII.2; GII.17; GII.14; GII.12). The following capsid types were detected for the first time by the NRC this year: GI.5.

Based on polymorphisms detected in the polymerase gene, 3 different P-types within gene group GI (GI.P7; GI.P2; GI.P3) are distinguished in Belgium in 2021. A total of 10 different P-types distinguished for gene group GII (GII.P31; GII.P8; GII.P12; GII.P4; GII.P17; GII.P21; GII.P16; GII.P7; GII.P30; GII.P31). Figure 3. shows that multiple genotypes are associated with several P-types, especially within the genogroup GII.

Most of the norovirus GII.4, GII.3, GII.7 strains were detected in children under the age of 2, whereas norovirus GII.6 and GII.17 cases were mainly associated with patients older than 65. The polymerase types GII.P12, GII.P4, GII.P21 and GII.P31 were detected in children under the age of 2 (see figure 4).



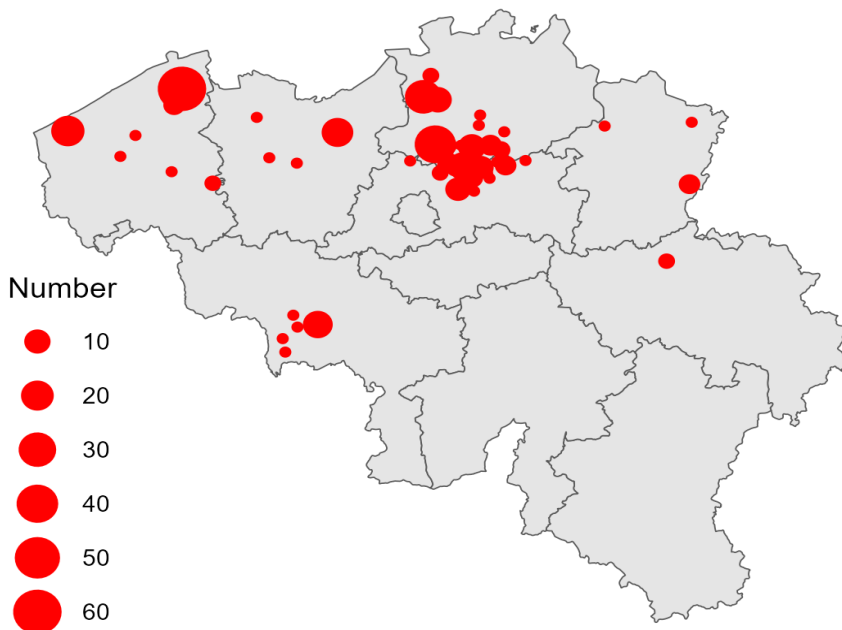
**Figure 3.** Norovirus capsid and polymerase dual type combinations



**Figure 4.** Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the age category of the patient in 2021.

# OUTBREAKS

## 1. General



**Figure 4.** Geographical distribution of the norovirus outbreaks in 2021, the size is determined by the number of cases.

The Service of foodborne pathogens of Sciensano houses both the NRC of Norovirus and the national reference laboratory (NRL) for foodborne outbreaks (FBO). In 2021 there were a total of 32 outbreak reports of acute gastroenteritis with a suspicion of norovirus infection. Among these, norovirus was identified in human samples from 22 outbreaks. Investigations into 12 of these acute gastroenteritis outbreaks indicated foodborne transmission as a suspected route of norovirus transmission to humans. The NRL FBO analysed the left-over food samples or when no longer available samples for the same batch for the presence of human pathogenic norovirus. In 2 of the outbreaks norovirus was detected in the suspected food products and a clear link with the food product was established. In 2 of these acute gastroenteritis outbreaks norovirus could only be detected in the human samples and a foodborne transmission could not be confirmed. Therefore, person-to-person transmission might have been the cause. 20 reported acute gastroenteritis outbreaks were not suspected to have arisen from foodborne transmission, with norovirus being detectable in 18 of these instances.

## 2. “... some more details”

Given the aftermath of the COVID-19 pandemic and the impact on the health inspection services, in 2021, only a total of 23 norovirus outbreaks were documented. Among these incidents, one occurred within a school environment, leading to the illness of 28 individuals. Subsequent investigation revealed that all affected individuals had consumed pasta salads prepared by a catering service. Further analysis identified the presence of norovirus genotype GII.P7 in both the afflicted individuals and two catering staff members who had exhibited gastrointestinal symptoms and were involved in food handling.

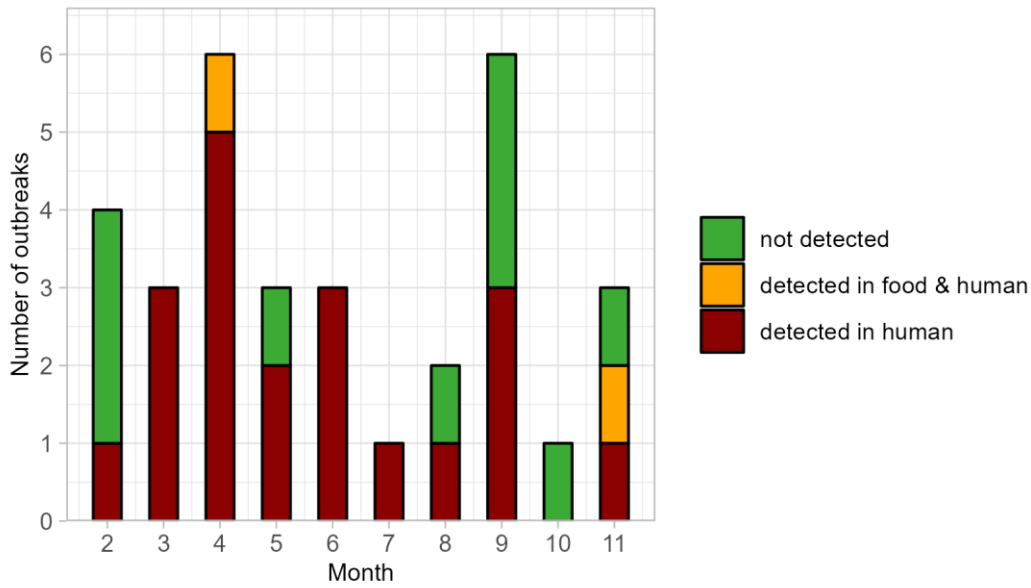
In another outbreak within a residential care home, evidence of norovirus presence was found in three separate environmental swabs.

Moreover, norovirus was implicated in co-infection outbreaks, including one involving *Bacillus cereus*. This outbreak occurred in a daycare facility where six children fell ill. Analysis of human samples detected the presence of both norovirus, sapovirus, and *B. cereus*. Interestingly, no norovirus was detected in any of the suspected food products.

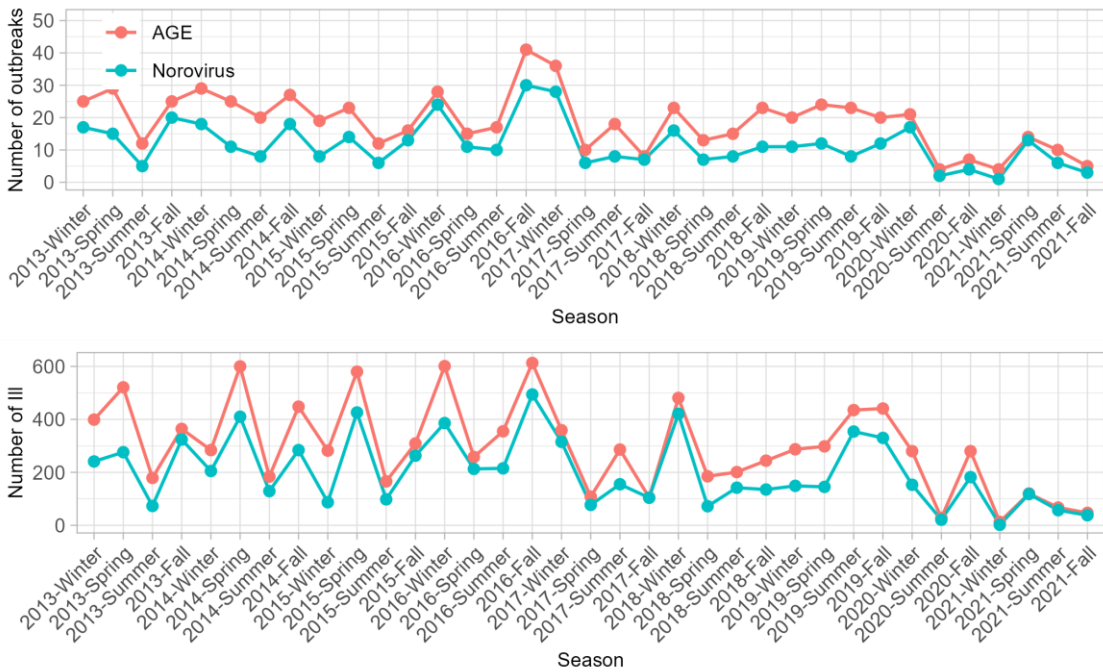
Furthermore, norovirus was associated with outbreaks primarily characterized by person-to-person transmission. For instance, during an outbreak in a camp, positive results for norovirus were obtained from environmental swabs taken from areas such as toilet seats with vomit or discharge residues, along with evidence of *Clostridium perfringens* and coagulase-positive staphylococcus presence.

Throughout 2021, Norovirus predominantly contributed to outbreaks characterized by person-to-person transmission. Notably, some outbreaks occurred within hospital settings, where affected individuals were already hospitalized at the time of infection acquisition.

Figure 5 illustrates the typical seasonal dynamics of norovirus, showcasing a prominent surge in outbreak occurrences during the fall-winter months (November-March). Probably due to the continued underreporting of acute gastroenteritis outbreaks no winter peak was observed in the data of 2021.



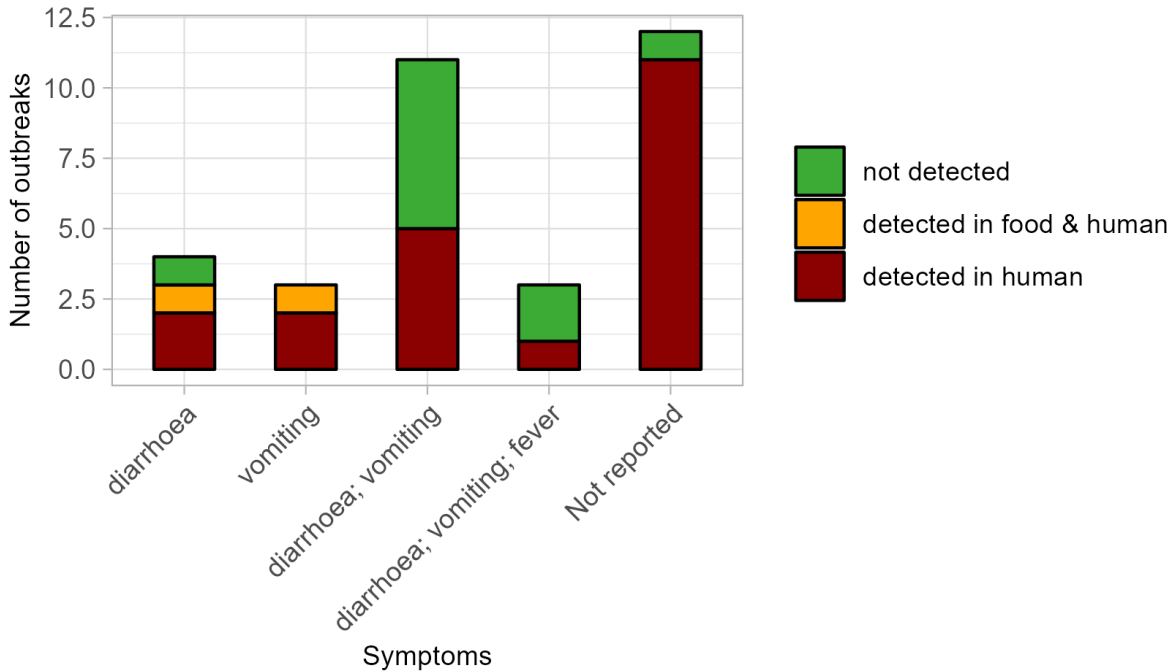
**Figure 5.** Number of acute gastroenteritis outbreaks reported to the NRC in 2021 per month.



**Figure 6.** Number of acute gastroenteritis (AGE) and norovirus outbreaks (top) and number of ill (bottom) reported to the NRC since 2013.

### 3. Symptoms

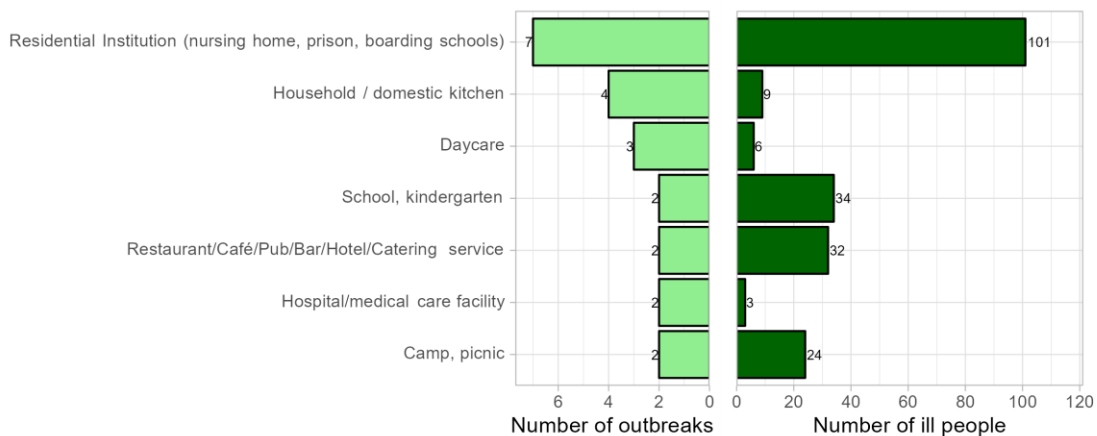
Most norovirus outbreaks were associated with diarrhoea; vomiting cases. The symptoms of the affected cases were not reported for 12 of the acute gastroenteritis outbreaks.



**Figure 7.** Reported symptoms associated with the AGE outbreaks reported to the NRC in 2021.

### 4. Setting

Norovirus outbreaks predominantly occurred in several key settings: residential institutions, camps and households. Also the outbreaks with the most reported cases took place in residential institutions. Notably, the most substantial norovirus outbreak on record for 2021 was in a nursing home encompassed 60 reported cases (figure 8).

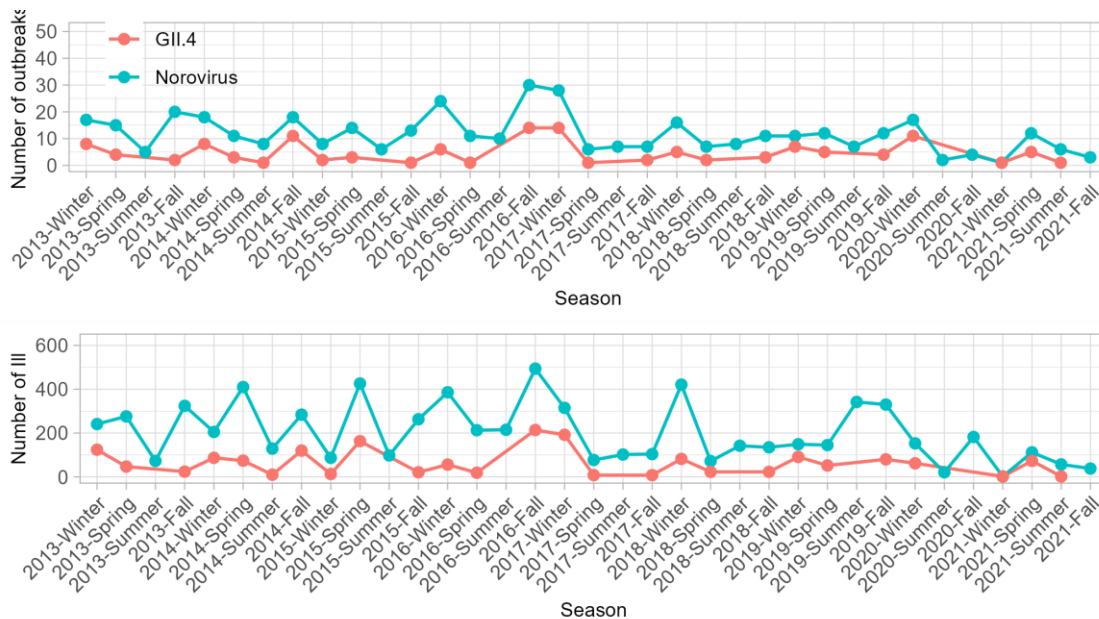


**Figure 8.** Number of norovirus outbreaks and ill per setting as reported to the NRC in 2021.

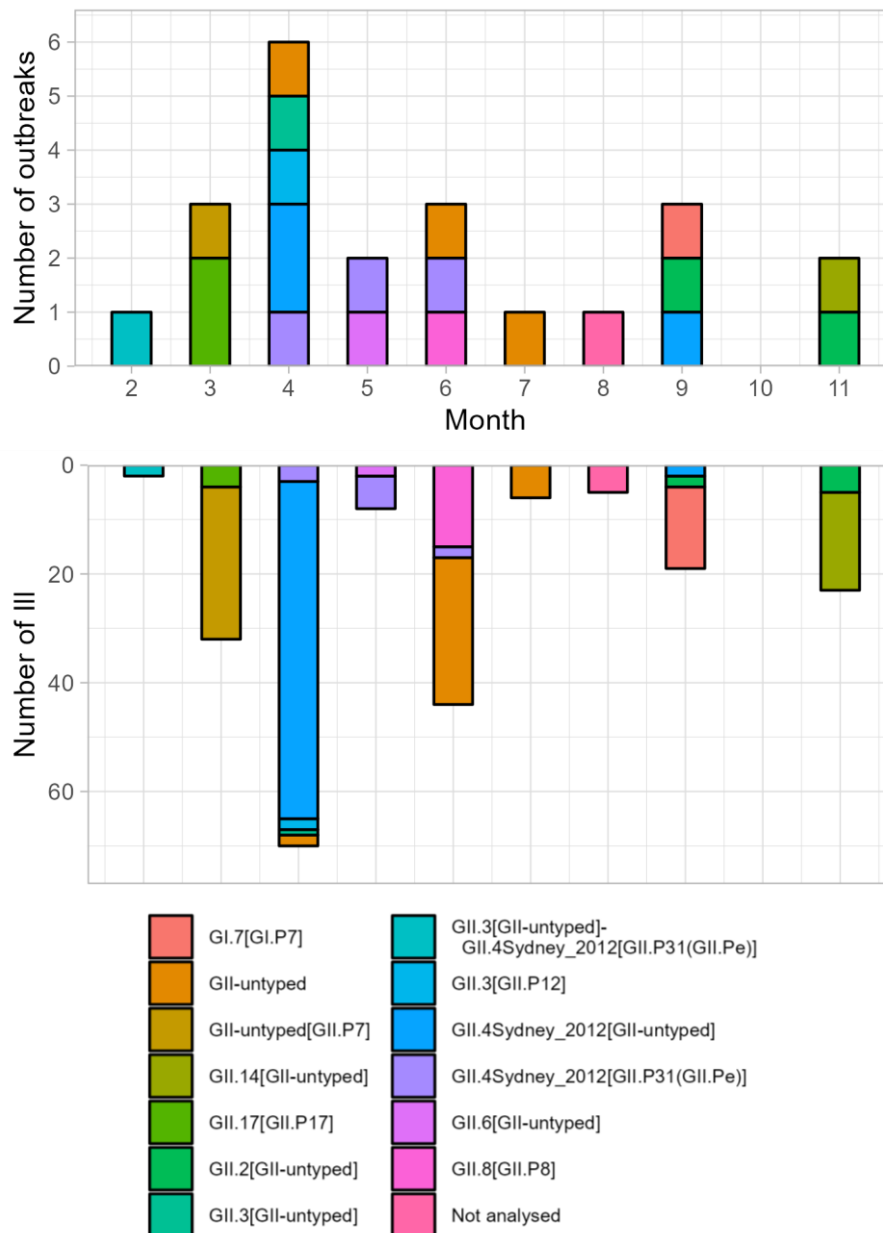
## 5. Genotypes

The norovirus capsid polymorphism that was often detected in outbreaks in 2021 was GII.4 Sydney 2012 and the most often detected polymerase was GII.P31 (figure 10). In the winter spanning 2015 to 2016, a novel GII.4 virus was reported, exhibiting resemblances to the pandemic GII.4 Sydney virus in the capsid region, yet featuring a distinctive polymerase sequence identified as GII.P16 (Cannon *et al.*, 2017). Subsequently, in November 2016, this variant was first identified in Belgium through a sporadic case. Since its initial detection, this particular P-type has steadily gained significance. However, the results of the NRC show that in 2021 most norovirus GII.4 Sydney 2012 were paired with GII.P31.

Figure 9 illustrates the temporal dynamics of Norovirus GII.4 outbreaks alongside the associated case counts since the start of the NRC activity in 2013. For the first time no GII.4 was detected for 6 months.



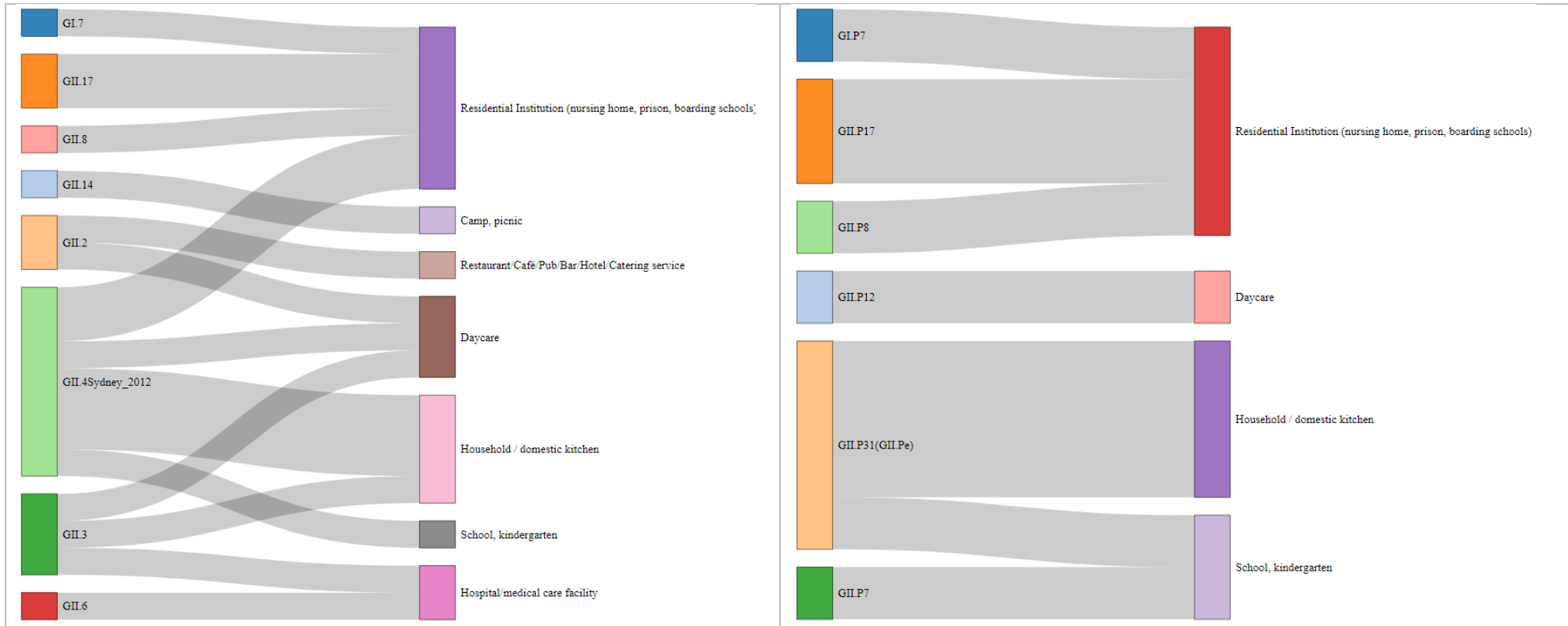
**Figure 9.** Number of norovirus and GII.4 outbreaks and ill reported to the NRC since the start of the NRC activity.



**Figure 10.** Number of norovirus outbreaks and ill per setting as reported to the NRC in 2021. “Not analysed” comprises all samples that were either not received by the NRC, contained low levels of norovirus, or experienced unsuccessful sequencing.

Norovirus infections in residential institutes mainly occurred by norovirus GII.4 Sydney 2012, GI.7[GI.P7], GII.17[GII.P17] and GII.8[GII.P8]. While norovirus GII.4 Sydney 2012 was also implicated in households and schools in the combination with GII.P31. The genotypes more related to foodborne outbreaks were norovirus GII.2, GII.3, GII.4 and GII.P7 (figure 11).





**Figure 11.** Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the setting of the norovirus outbreaks in 2021.

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