NATIONAL REFERENCE CENTRE NOROVIRUS

Annual report 2022

WHO WE ARE

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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With support of

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EXECUTIVE SUMMARY

In 2022, the National Reference Centre (NRC) received reports of 47 outbreaks potentially linked to Norovirus, affecting a minimum of 1349 individuals. Confirmation of Norovirus as the causative agent was established in 28 of these outbreaks, affecting a minimum of 1004 individuals. Subsequent typing via sequencing allowed determination of Norovirus genogroup and genotype in 23 outbreaks, while technical limitations precluded such determination in the remaining 5 cases. Among the typed outbreaks, Norovirus genogroup GI was identified in 7 instance, while genogroup GII was predominant, detected in 20 outbreaks.

Of the reported outbreaks in 2022, 3 were suspected to involve Norovirus transmission through food. Norovirus was detected in human samples in all of these outbreaks Specifically, in 1 outbreak, 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 restaurants.

In 2022, the predominant circulating strain of Norovirus remained GII.4 (Sydney 2012) with 4 outbreaks, initially identified in September 2012 by van Beek et al. Additionally, Norovirus genotype GI.3 was implicated in 3 outbreaks during this period.

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OVERVIEW OF ACTIVITIES

1. Norovirus detection

In 2022, the NRC Norovirus received a total of 292 human samples (see table 1). Diagnostic detection of human pathogenic Norovirus by reverse transcriptase (RT-)PCR was conducted on 269 of these samples, revealing the presence of the virus in 192 cases. Additionally, 23 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 2022

	Samples received
Total	292
Norovirus detected	215
Norovirus not detected	77
Outbreak	67
Sporadic	148

A total of 222 samples tested positive for norovirus. Among these, 67 were identified during an outbreak, while the remaining 148 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 2022 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=15).

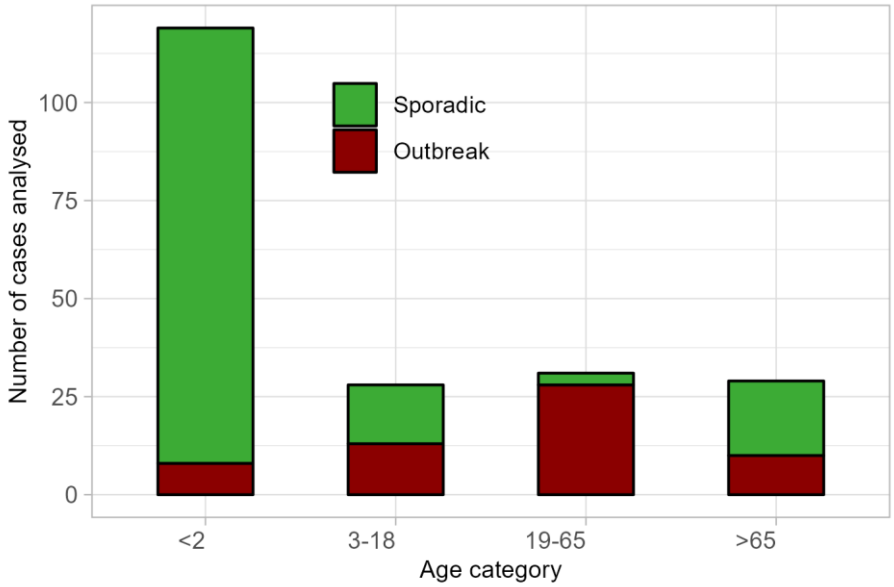

Figure 1. Norovirus detected in 2022 per age category.

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*., 2022).

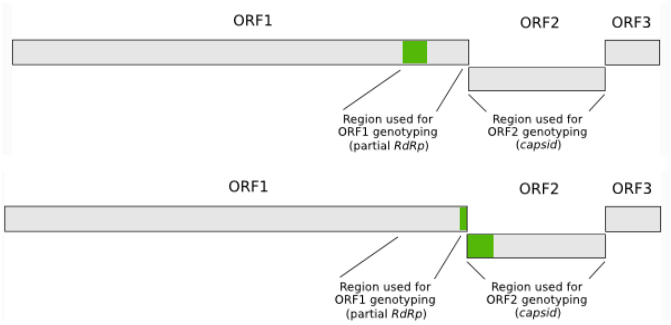

Figure 2. Schematic representation of the location of genomic regions used for genotyping of Norovirus (Vinjé et al 2004).

A total of 191 samples underwent genotyping, with the NRC successfully sequencing either the capsid region, the polymerase region, or both for 185 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 191 samples, 60 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 11 of the samples contained the human pathogenic genogroup GI, 27 the human pathogenic genogroup GII and 19 both. The remaining 131 samples originated from sporadic cases. The serogrouping assay showed that 21 of the samples contained the human pathogenic genogroup GI, 99 the human pathogenic genogroup GII and 3 both.

Based on polymorphisms detected in the capsid gene, 5 different genotypes within genogroup GI (GI.2; GI.3; GI.4; GI.5; GI.6) are distinguished in Belgium in 2022. A total of 11 different genotypes distinguished for genogroup GII (GII.2; GII.3; GII.4 Sydney_2012; GII.4; GII.5; GII.6; GII.7; GII.12; GII.14; GII.13; GII.17).
Based on polymorphisms detected in the polymerase gene, 6 different P-types within gene group GI (GI.P3; GI.P2; GI.P5; GI.P11; GI.P7; GI.P4) are distinguished in Belgium in 2022. A total of 8 different P-types distinguished for gene group GII (GII.P2; GII.P4; GII.P7; GII.P12; GII.P16; GII.P17; GII.P31; GII.P30). The following polymerase types were detected for the first time by the NRC this year: GI.P11 . Figure 3. shows that multiple genotypes are associated with several P-types, especially within the genogroup GII.

Most of the norovirus GII.3, GII.4, GII.6, GII.7; GII.P12 and GII.P7 strains were detected in children under the age of 2, whereas norovirus GI.6, GII.2, GI.P11, GI.P7 cases were mainly associated with patients between 19 and 65 years of age. (see figure 4).

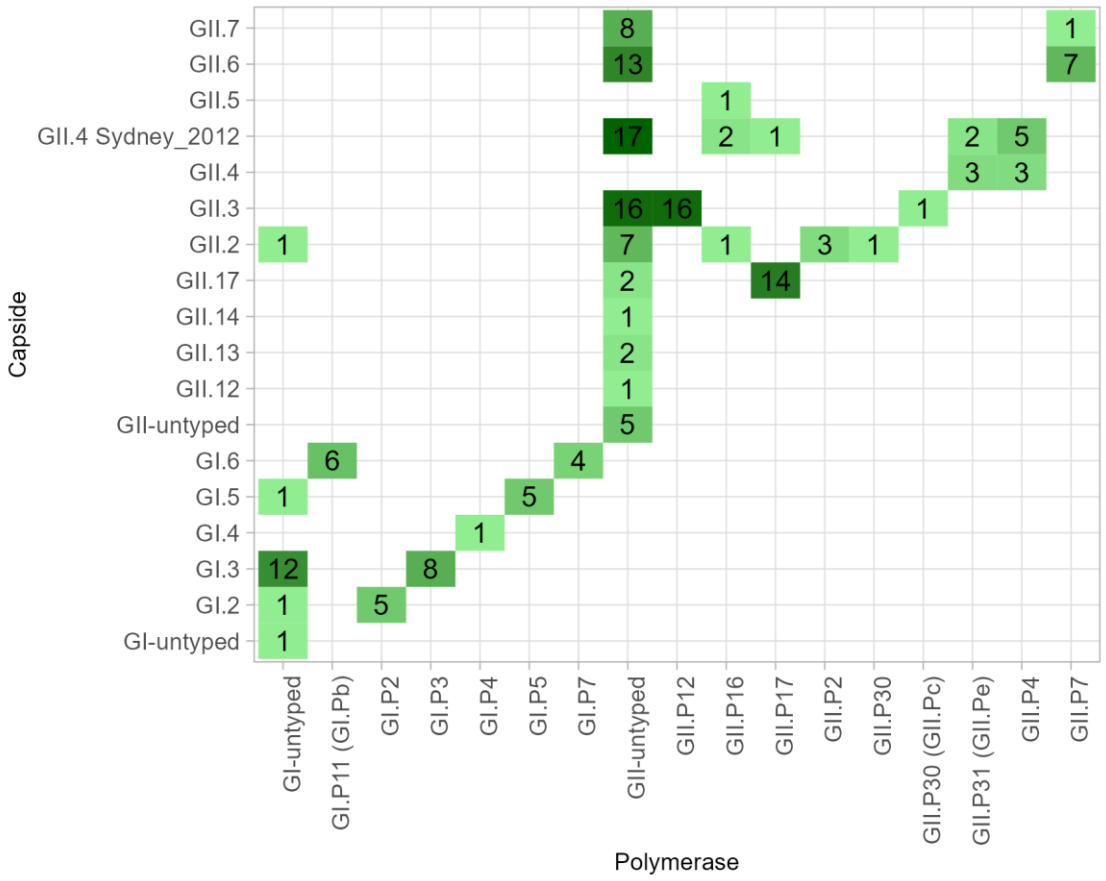

Figure 3. Norovirus capsid and polymerase dual type combinations

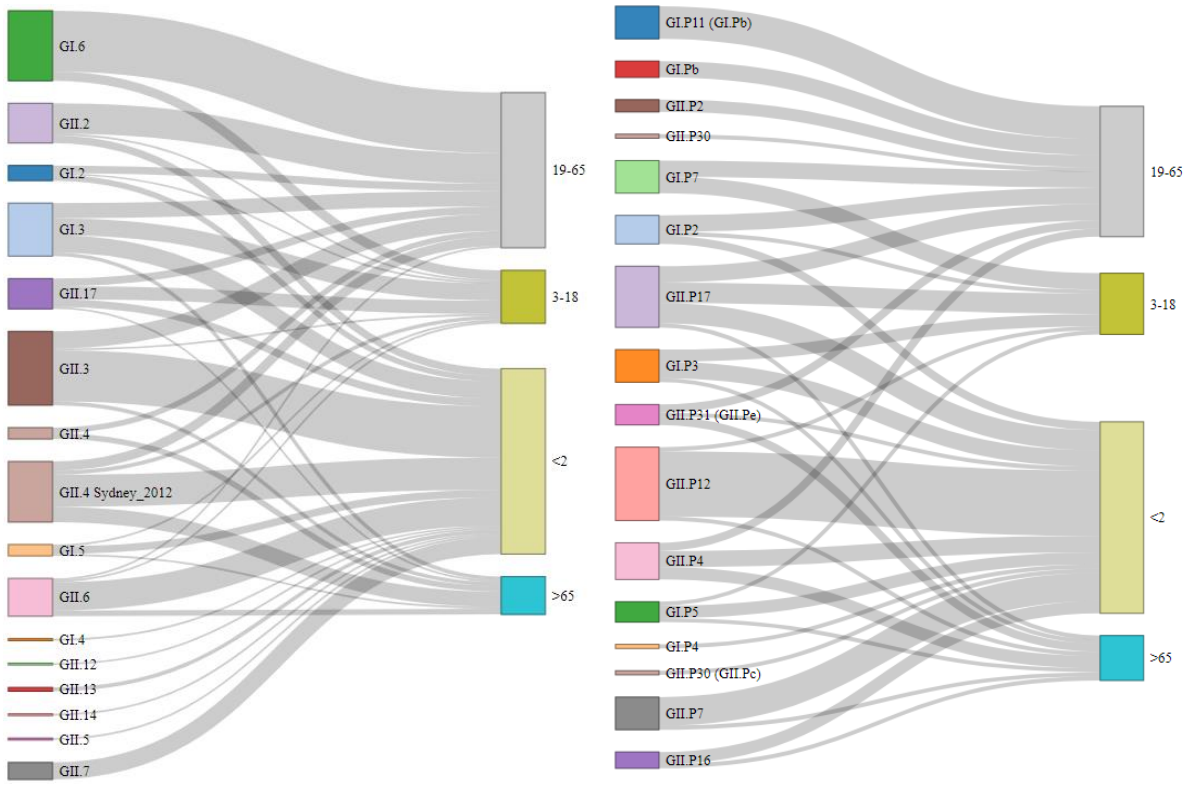

Figure 4. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the age category of the patient in 2022.

OUTBREAKS

1. General

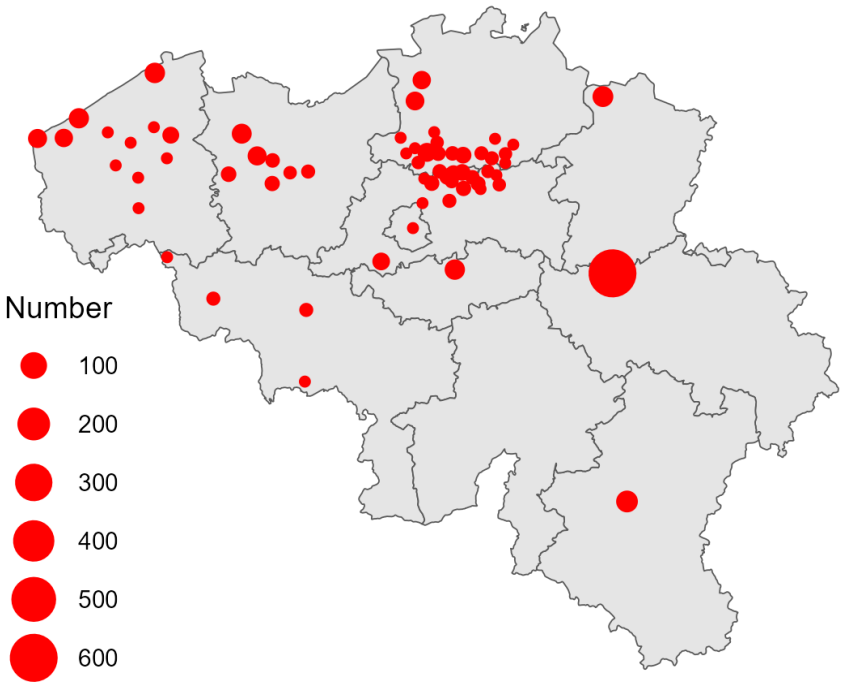

Figure 4. Geographical distribution of the norovirus outbreaks in 2022, the size is determined by the number of cases.

The Service of foodborne pathogens of Sciensano houses both the NRC of Norovirus and the national reference laboratory (NRL) for foodborne outbreaks (FBO). In 2022 there were a total of 47 outbreak reports of acute gastroenteritis with a suspicion of norovirus infection. Among these, norovirus was identified in human samples from 28 outbreaks. Investigations into 10 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 1 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.
37 reported acute gastroenteritis outbreaks were not suspected to have arisen from foodborne transmission, with norovirus being detectable in 25 of these instances.

2. “… some more details”

Several outbreaks were investigated suspected of norovirus infection. Three of these outbreaks were associated with school activities: two school trips to the seaside (Lombardsijde and Koksijde) and an informational evening (Lievegem). Norovirus was detected in human samples in three of these cases, yet remained undetected in both food and environmental samples. The NRC successfully characterized the noroviruses involved in the Koksijde sea class outbreak as GII.7[GII.P17] and the Lievegem informational evening outbreak as GII.4[GII.P4].
A norovirus epidemic affected six cycling tourists who had stayed at a staycation center, exhibiting symptoms of vomiting. Two food handlers displayed similar symptoms, and one handler's child also fell ill. Norovirus GII was detected in the child's faeces and on a cutting board surface. Sequencing analysis identified GII.12 in the child's faecal sample, although the viral load in swab samples was too low for sequencing. This outbreak was deemed a strongly supported case due to the epidemiological link between tourists and ill food handlers, coupled with norovirus GII detection in both the cutting board swab and the child's feces.

The most significant outbreak occurred in Hodeige, Wallonia, where over 600 people fell ill after consuming tap water contaminated with sewage. Analysis of twenty fecal samples and one vomit sample revealed the presence of Norovirus GI and GII (17 out of 20 samples), astrovirus (13/20 samples), sapovirus (8/20 samples), and other bacterial agents (Campylobacter in at least one person). An epidemiological survey involving 397 participants (representing 35% of the region's population) indicated a peak incidence between October 24th and 27th, following the discovery of tap water issues by residents. Norovirus sequencing revealed significant diversity among the 17 positive samples.

Norovirus was additionally implicated in co-infection with *C. perfringens* in one outbreak. Throughout 2022, 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 seasonal dynamics of norovirus, a typical prominent surge in outbreak occurrences during the fall-winter months (November-March) are no longer observed in the NRC data of 2022.

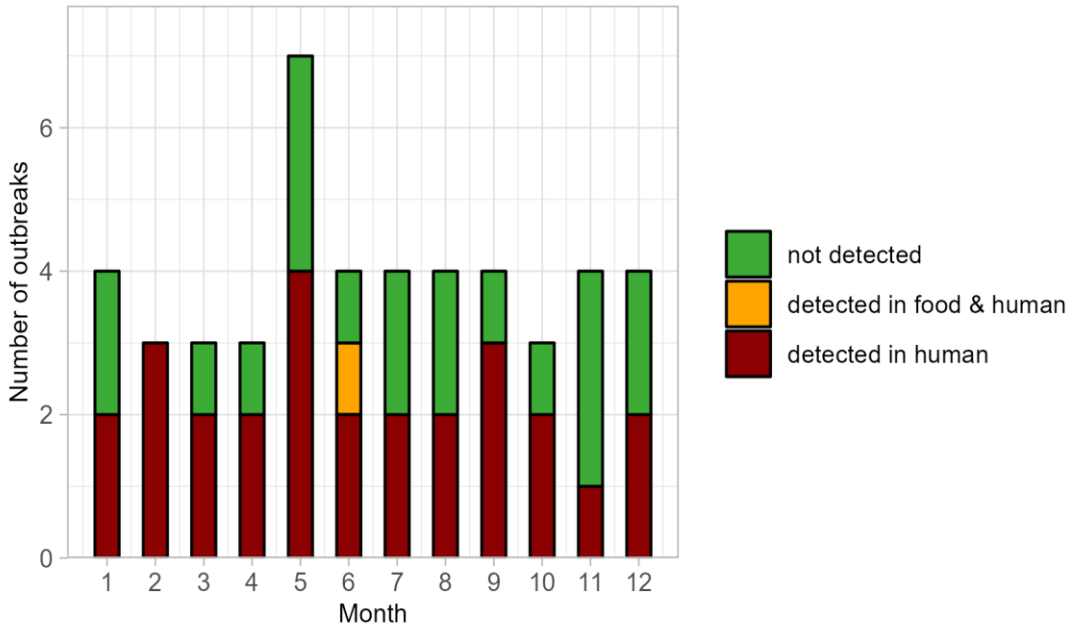

Figure 5. Number of acute gastroenteritis outbreaks reported to the NRC in 2022 per month.

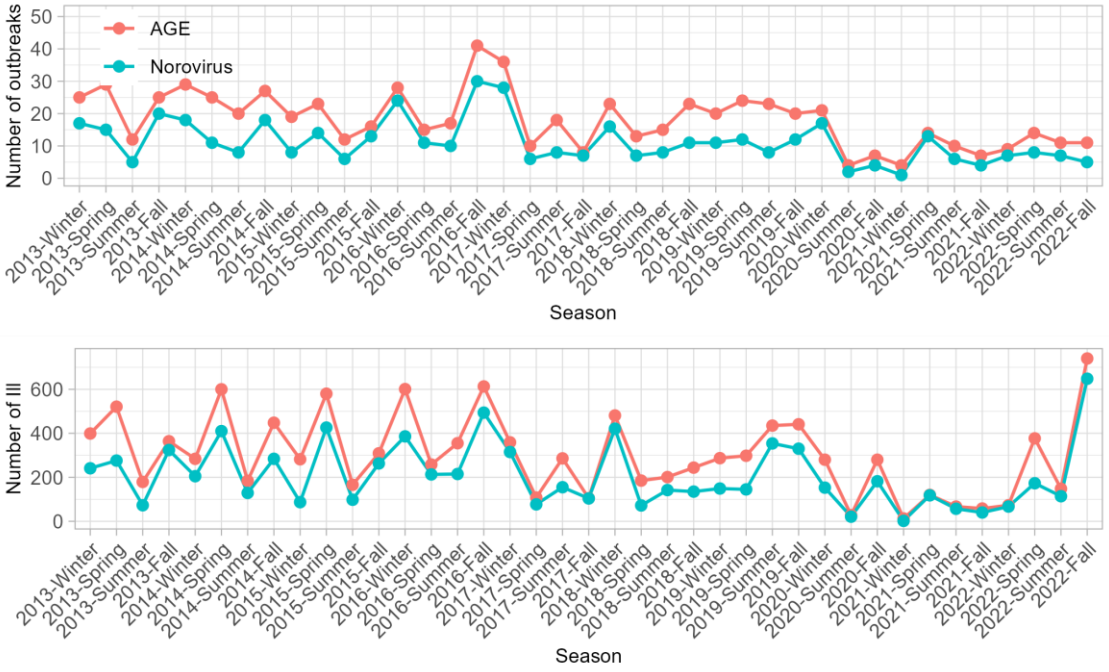

Figure 6. Number of acute gastroenteritis (AGE) and norovirus outbreaks (top) and number of ill (bottom) reported to the NRC since 2013.

3. Symptoms

Most norovirus outbreaks were associated with diarrhoea; vomiting cases. The symptoms of the affected cases were not reported for 20 of the acute gastroenteritis outbreaks.

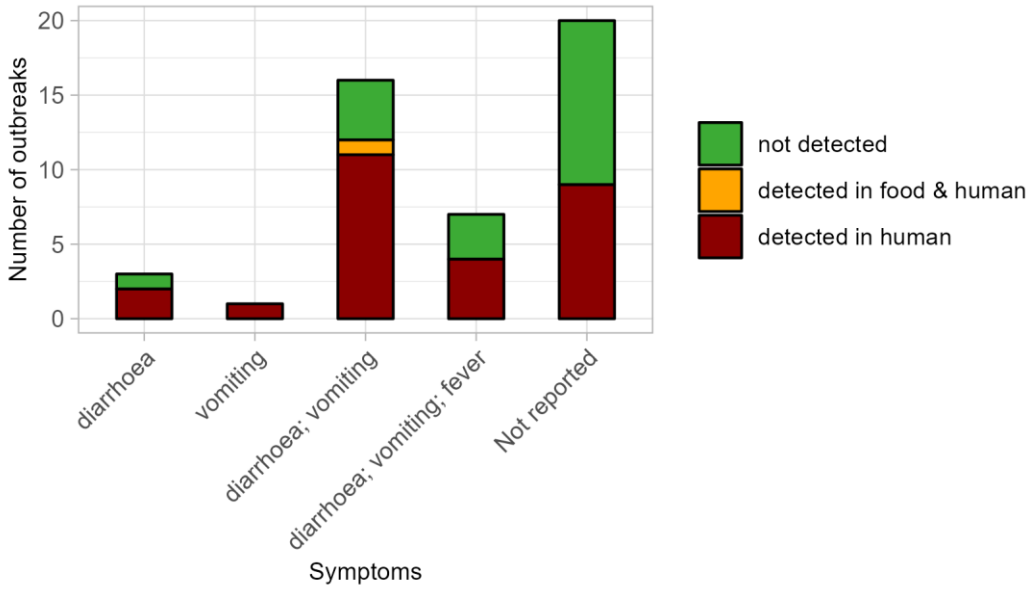

Figure 7. Reported symptoms associated with the AGE outbreaks reported to the NRC in 2022.

4. Setting

Norovirus outbreaks predominantly occurred in several key settings: residential institutions, camps and restaurants. Also the outbreaks with the most reported cases took place in residential institutions. Notably, the most substantial norovirus outbreak on record for 2022 taak place in multiple places linked to the contamination of drinking water encompassed >600 reported cases (figure 8).

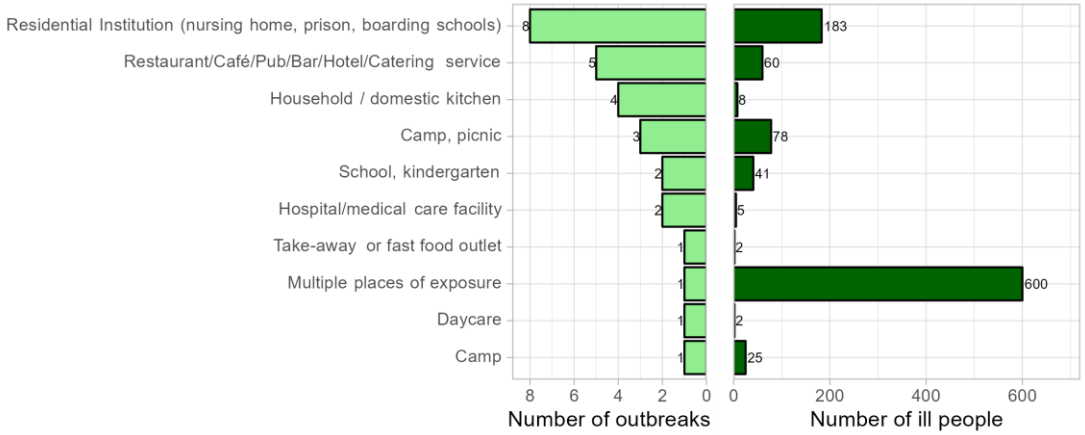

Figure 8. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2022.

5. Genotypes

The norovirus capsid polymorphism that was often detected in outbreaks in 2022 was GII.4 Sydney 2012 and the most often detected polymerase was GII.P17 (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 2022 most norovirus GII.4 Sydney 2012 were paired with GII.P4.

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. The number of outbreaks linked to Norovirus GII.4 remained stable throughout 2022.

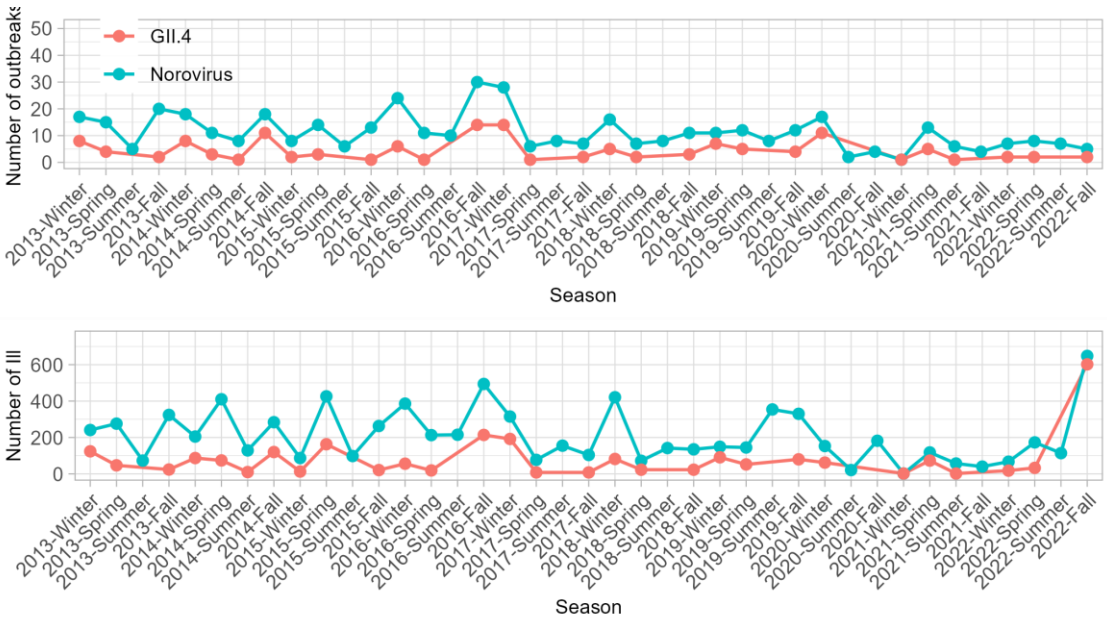

Figure 9. Number of norovirus and GII.4 outbreaks and Ill reported to the NRC since the start of the NRC activity.

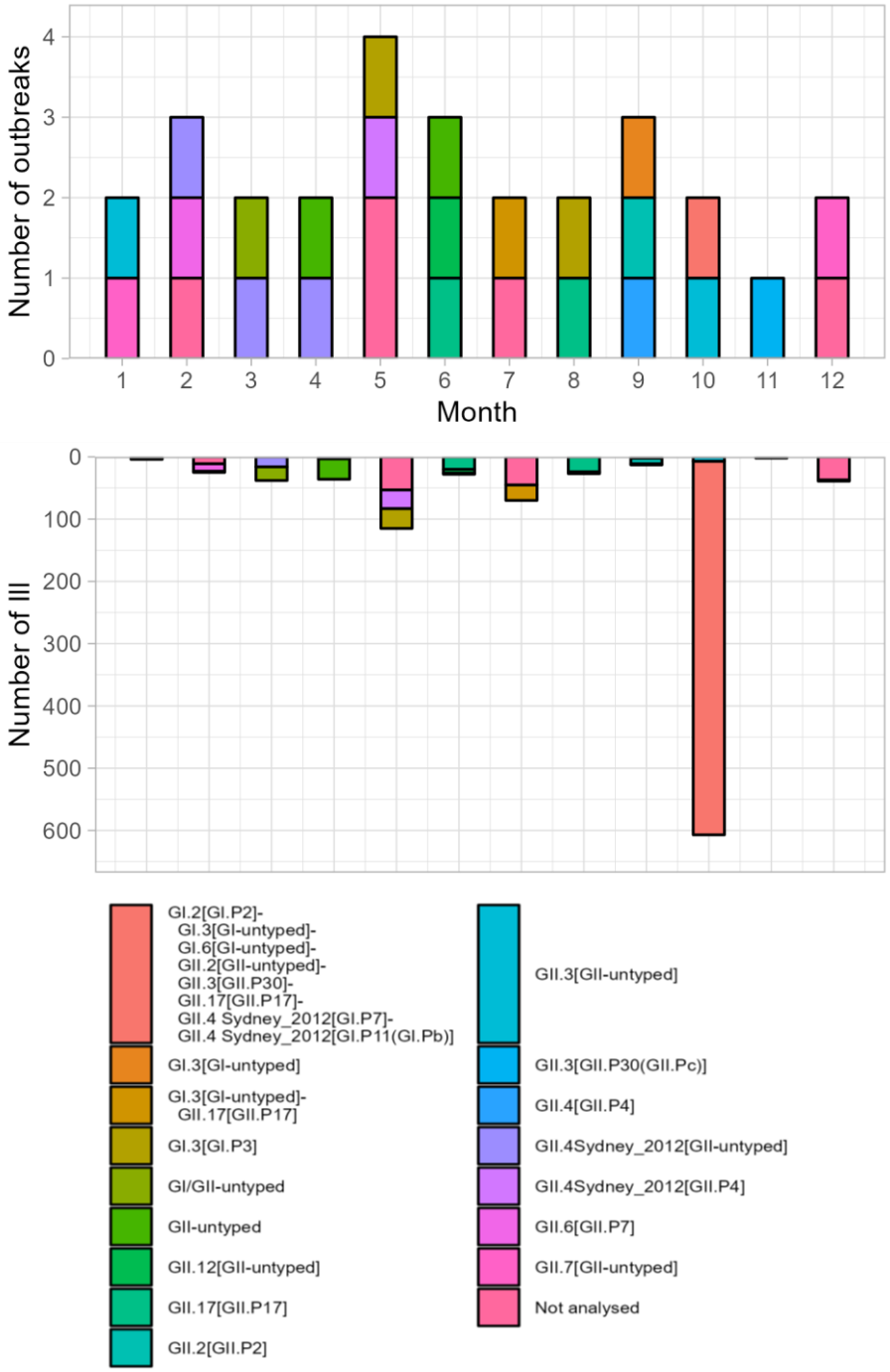

Figure 10. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2022. “Not analysed” comprises all samples that were either not received by the NRC, contained low levels of norovirus, or experienced unsuccessful sequencing.

Norovirus infections in residential institutes mainly occurred by norovirus GII.4 Sydney 2012, GI.3, GII.12 and GII.6 in the combination with GII.P7 and GI.P3. While norovirus GII.4 and GI.3 were also implicated in hospitals and medical facilities in the combination with GII.P4 and GI.P3 (figure 11).

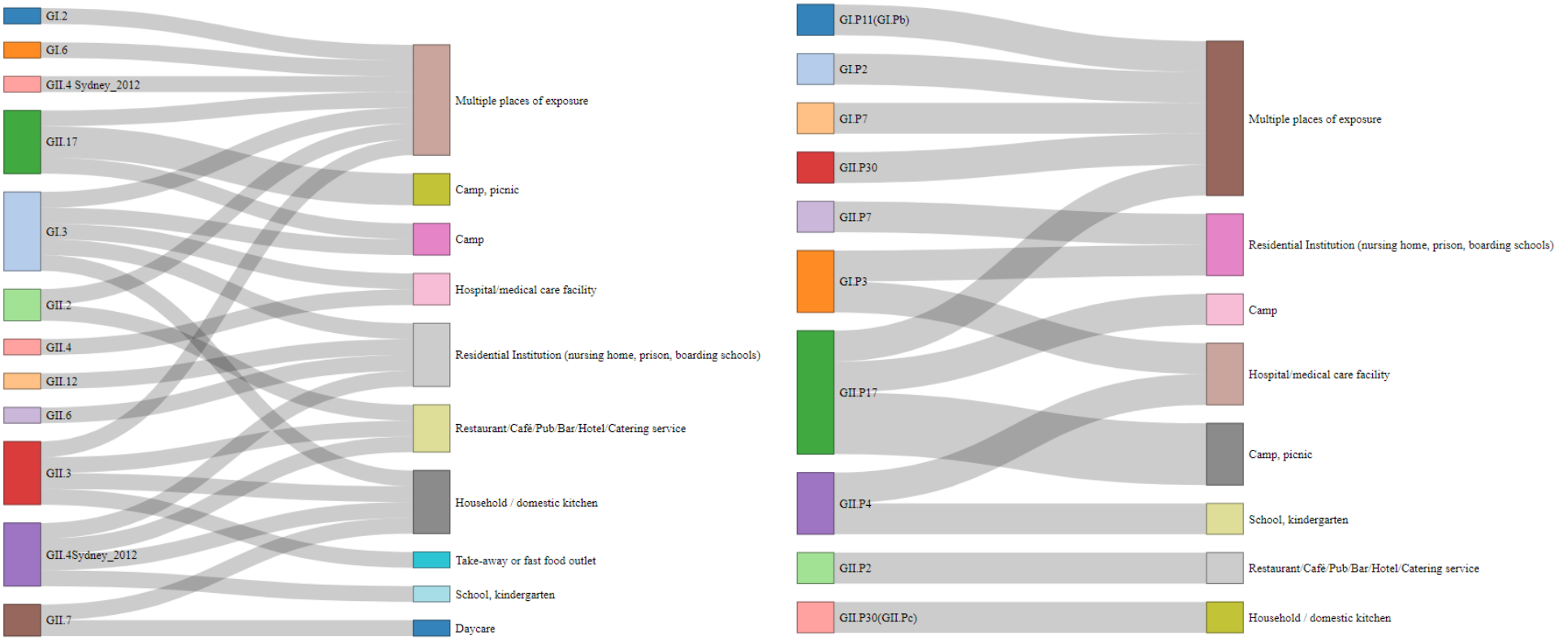

Figure 11. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the setting of the norovirus outbreaks in 2022.

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