NATIONAL REFERENCE CENTRE NOROVIRUS

Annual report 2020

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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EXECUTIVE SUMMARY

In 2020, the National Reference Centre (NRC) received reports of 32 outbreaks potentially linked to Norovirus, affecting a minimum of 588 individuals. Confirmation of Norovirus as the causative agent was established in 23 of these outbreaks, affecting a minimum of 356 individuals. Subsequent typing via sequencing allowed determination of Norovirus genogroup and genotype in 18 outbreaks, while technical limitations precluded such determination in the remaining 5 cases. Among the typed outbreaks, Norovirus genogroup GI was identified in 3 instances, while genogroup GII was predominant, detected in 20 outbreaks.

Of the reported outbreaks in 2020, 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 hospital and medical care facility.

In 2020, the predominant circulating strain of Norovirus remained GII.4 (Sydney 2012) with 11 outbreaks, initially identified in September 2012 by van Beek et al. Additionally, Norovirus genotype GII.2 was implicated in 2 outbreaks during this period.

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OVERVIEW OF ACTIVITIES

1. Norovirus detection

In 2020, the NRC Norovirus received a total of 129 human samples (see table 1). Diagnostic detection of human pathogenic Norovirus by reverse transcriptase (RT-)PCR was conducted on 111 of these samples, revealing the presence of the virus in 75 cases. Additionally, 18 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 2020

	Samples received
Total	129
Norovirus detected	93
Norovirus not detected	36
Outbreak	46
Sporadic	47

A total of 93 samples tested positive for norovirus. Among these, 46 were identified during an outbreak, while the remaining 47 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) and the oldest age category (>65). Remarkably, the number of cases linked to outbreaks was significantly higher in the eldest category. In the age category of 19-65, almost all positive samples were associated with outbreaks. It is worth noting that for some positive samples, birth dates were not provided, leading to their exclusion from the analysis (n=6).

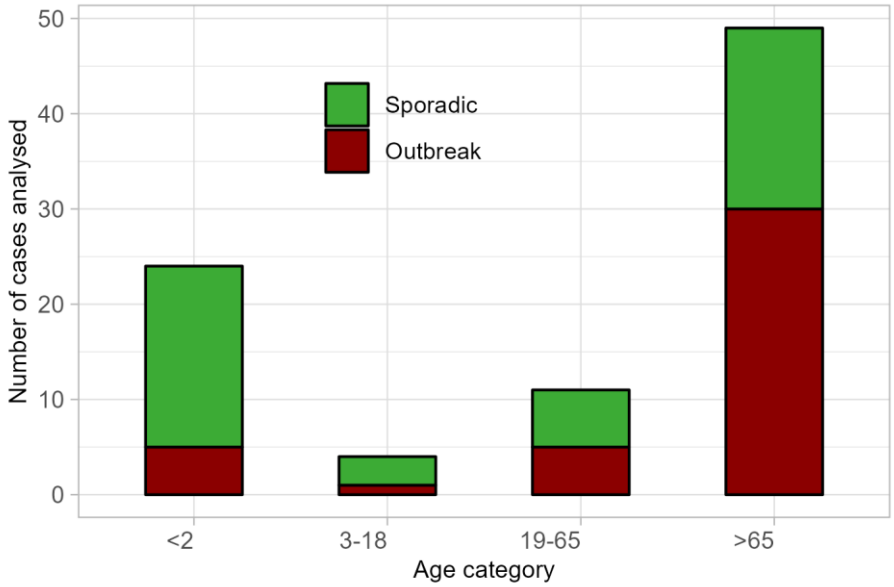

Figure 1. Norovirus detected in 2020 per age category.

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*., 2020).

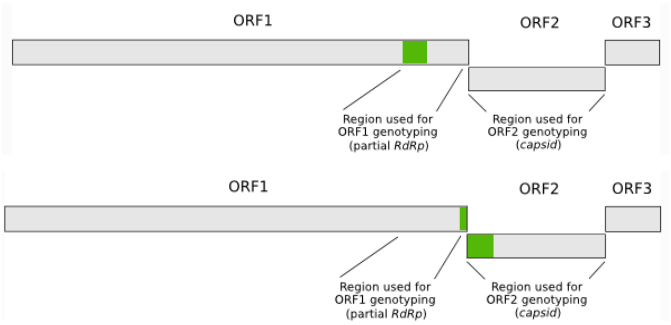

Figure 2. Schematic representation of the location of genomic regions used for genotyping of Norovirus (Vinjé et al 2004).

A total of 82 samples underwent genotyping, with the NRC successfully sequencing either the capsid region, the polymerase region, or both for 75 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 in 3 of the cases, 4 were detected by the clinical laboratory by RT-PCR.

Among the 82 samples, 38 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, 32 the human pathogenic genogroup GII and 3 both. The remaining 44 samples originated from sporadic cases. The serogrouping assay showed that 2 of the samples contained the human pathogenic genogroup GI, 25 the human pathogenic genogroup GII and 4 both.

Based on polymorphisms detected in the capsid gene, 3 different genotypes within genogroup GI (GI.2; GI.1; GI.P7) are distinguished in Belgium in 2020. A total of 9 different genotypes distinguished for genogroup GII (GII.4 Sydney 2012; GII.2; GII.3; GII.17; GII.6; GII.7; GII.4; GII.10; GII.12). The following capsid types were detected for the first time by the NRC this year: GII.10 and GI.P7.
Based on polymorphisms detected in the polymerase gene, 2 different P-types within gene group GI (GI.P2; GI.P1) are distinguished in Belgium in 2020. A total of 5 different P-types distinguished for gene group GII (GII.P16; GII.P17; GII.P30; GII.P7; GII.P12). The following polymerase types were detected for the first time by the NRC this year: GI.P1. Figure 3. shows that multiple genotypes are associated with several P-types, especially within the genogroup GII.

Most of the norovirus GII.4, GII.10, GII.6 strains were detected in patients older than 65, whereas only a small portion of norovirus GII.2 cases were associated with this age category. The latter type was mainly found in children under the age of 2, like GII.3, GII.7 and GI.2. As last year a portion of norovirus GII.4 could be detected in the children under the age of 2 years of age. The polymerase types GII.P16 and GII.P17 associated with GII.4 were detected in patients older than 65 (see figure 4).

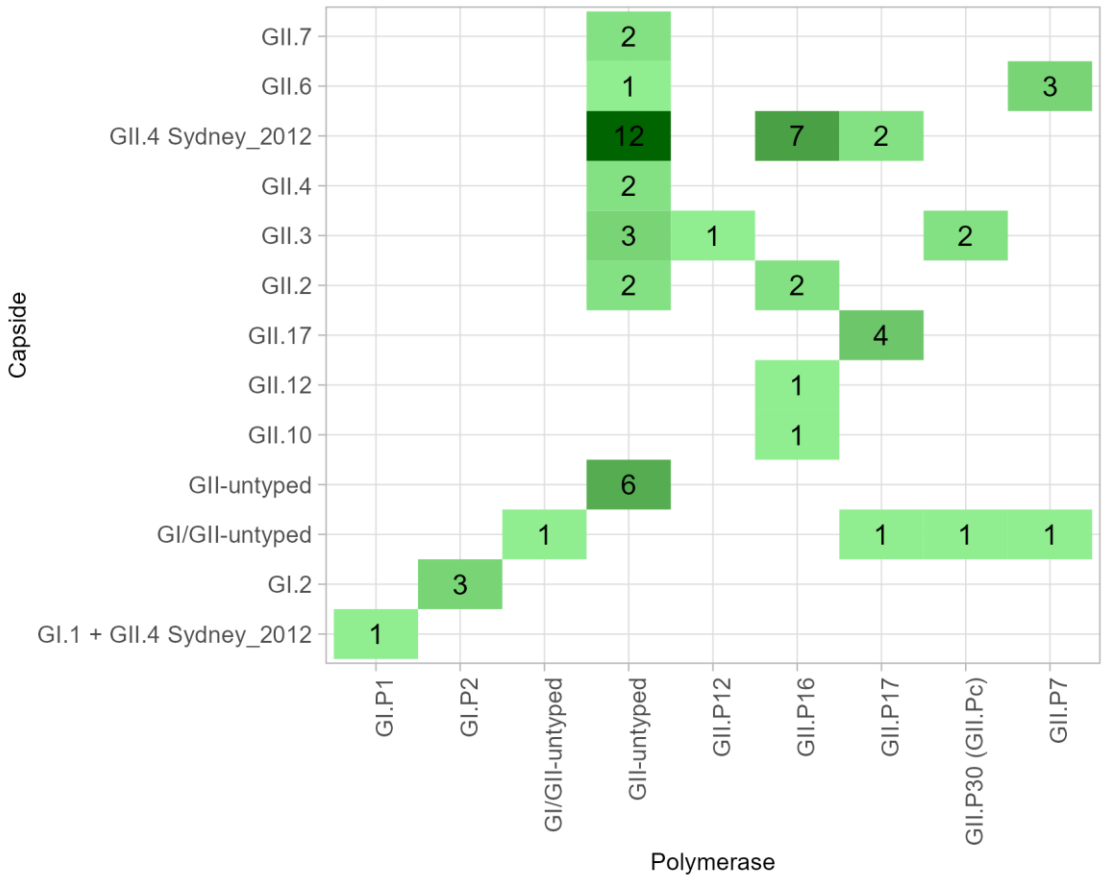

Figure 3. Norovirus capsid and polymerase dual type combinations

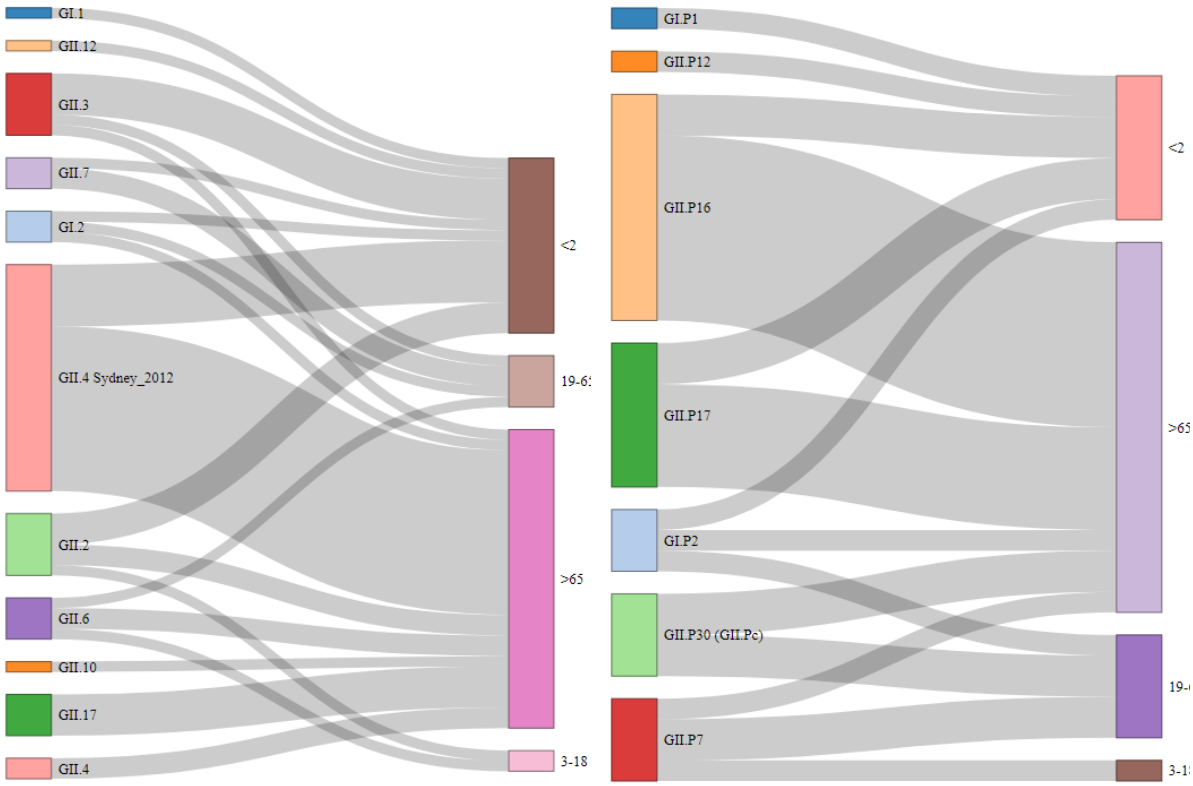

Figure 4. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the age category of the patient in 2020.

OUTBREAKS

1. General

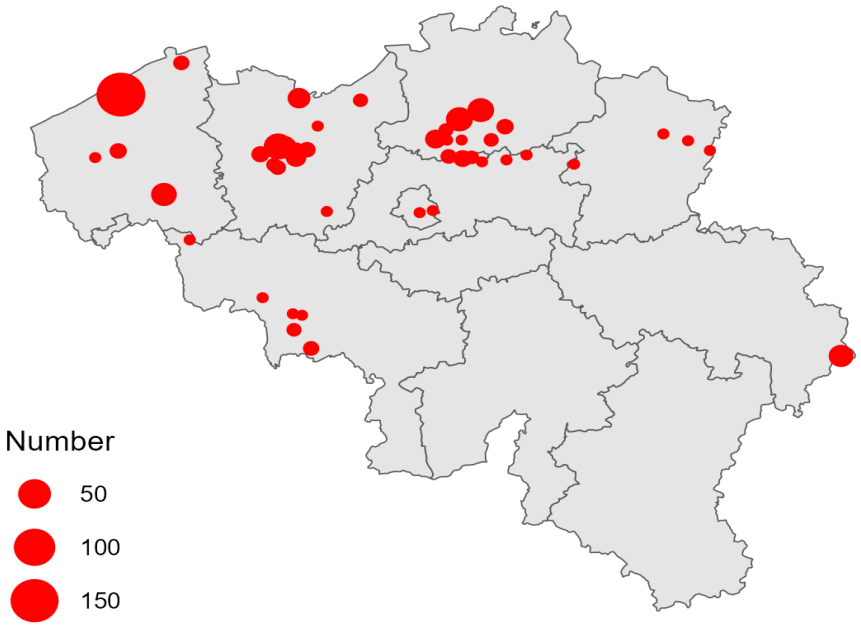

Figure 4. Geographical distribution of the norovirus outbreaks in 2020

The Service of foodborne pathogens of Sciensano houses both the NRC of Norovirus and the national reference laboratory (NRL) for foodborne outbreaks (FBO). In 2020 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 23 outbreaks. Investigations into 11 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.
21 reported acute gastroenteritis outbreaks were not suspected to have arisen from foodborne transmission, with norovirus being detectable in 20 of these instances.

2. “… some more details”

In 2020, there was a significant decrease in the number of reported outbreaks, likely attributed to the implementation of lockdown measures in response to the COVID-19 pandemic. Additionally, the health inspection services in Belgium redirected their resources towards managing the pandemic, resulting in a steep decrease of outbreak reports to the NRC.

In 2020, a notable outbreak of Norovirus occurred in a school, affecting 151 individuals. Typing confirmed the presence of Norovirus genotype GII.7[GII.P7] in both human cases and the vegetables consumed during the outbreak. Notably, Norovirus has been implicated in outbreaks characterized by person-to-person transmission.
Throughout 2020, 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). While a winter peak was observed in the data of 2020, this was mainly due to the onset of the COVID-19 measures starting in March and the lack of reporting in the following months.

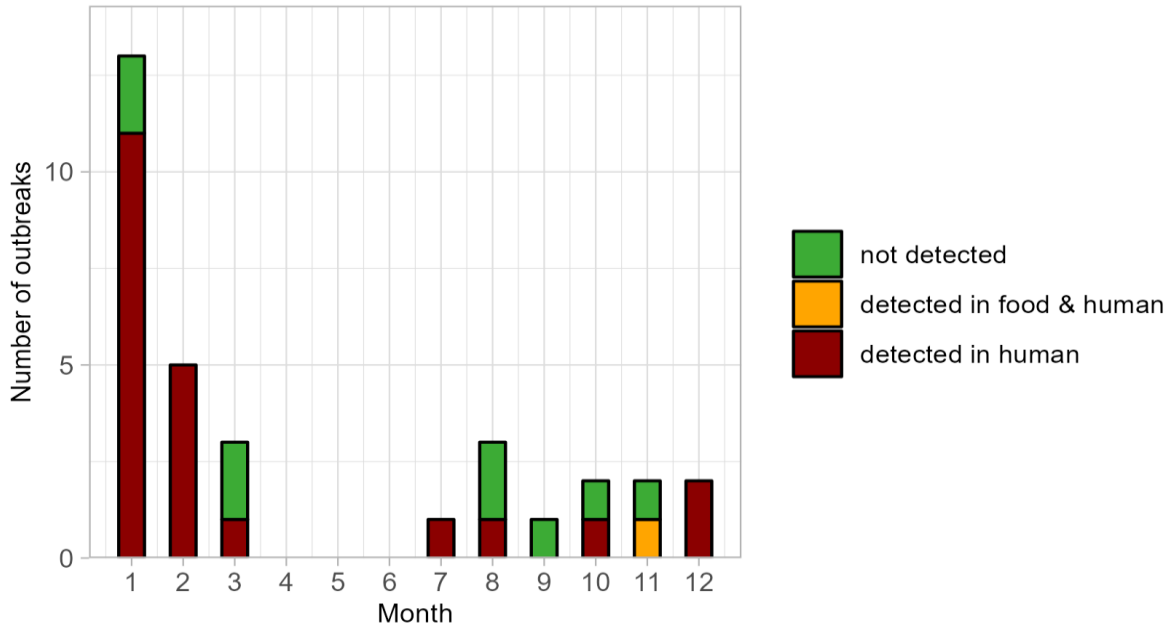

Figure 5. Number of acute gastroenteritis outbreaks reported to the NRC in 2020 per month.

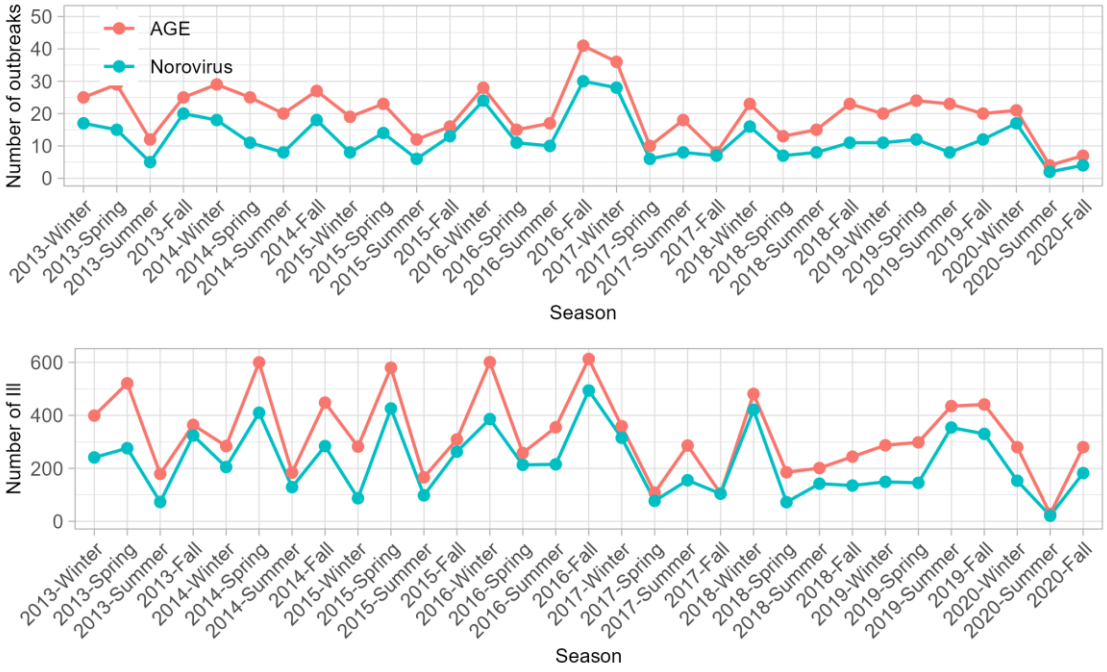

Figure 6. Number of acute gastroenteritis (AGE) and norovirus outbreaks (top) and number of ill (bottom) reported to the NRC since 2013.

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.

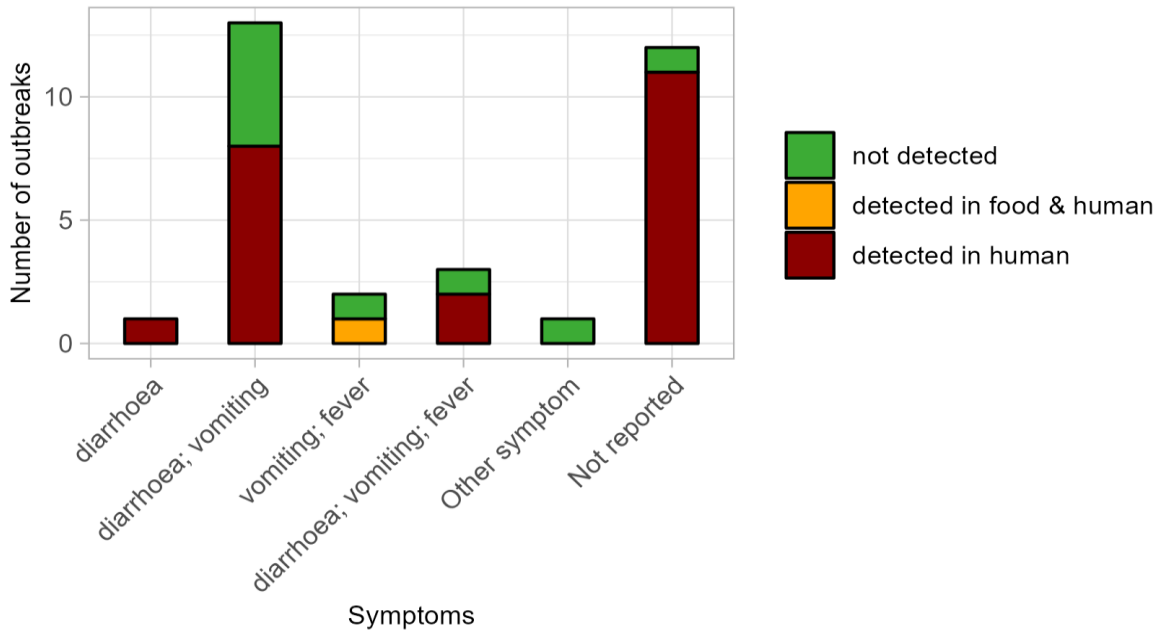

Figure 7. Reported symptoms associated with the AGE outbreaks reported to the NRC in 2020.

4. Setting

Norovirus outbreaks predominantly occurred in several key settings: residential institutions, camps and hospitals and medical care facilities. The outbreaks with the most reported cases took place in schools. Notably, the most substantial norovirus outbreak on record for 2020 was in a school encompassed 151 reported cases (figure 8).

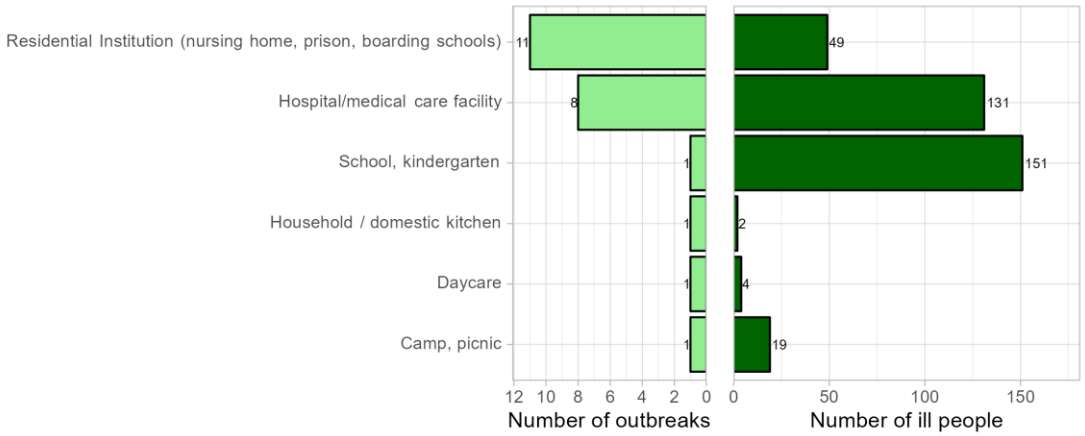

Figure 8. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2020.

5. Genotypes

The norovirus capsid polymorphism that was often detected in outbreaks in 2020 was GII.4 Sydney 2012 and the most often detected polymerase was GII.P16 (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, it is the second year in a row as the most frequently detected P-type by the NRC.

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.

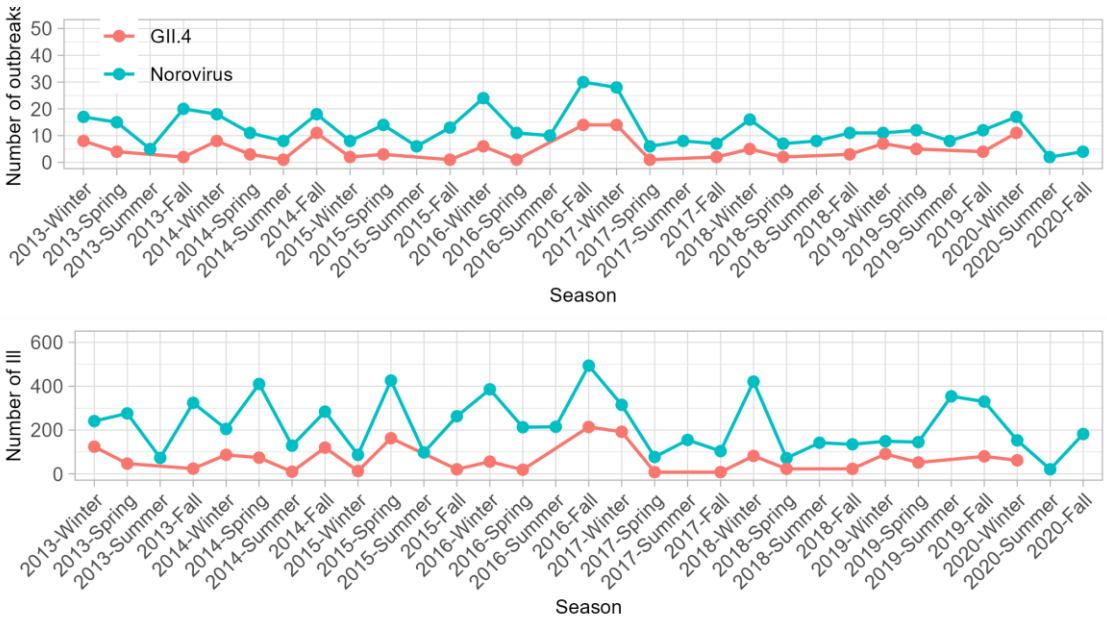

Figure 9. Number of norovirus and GII.4 outbreaks and Ill reported to the NRC since the start of the NRC activity.

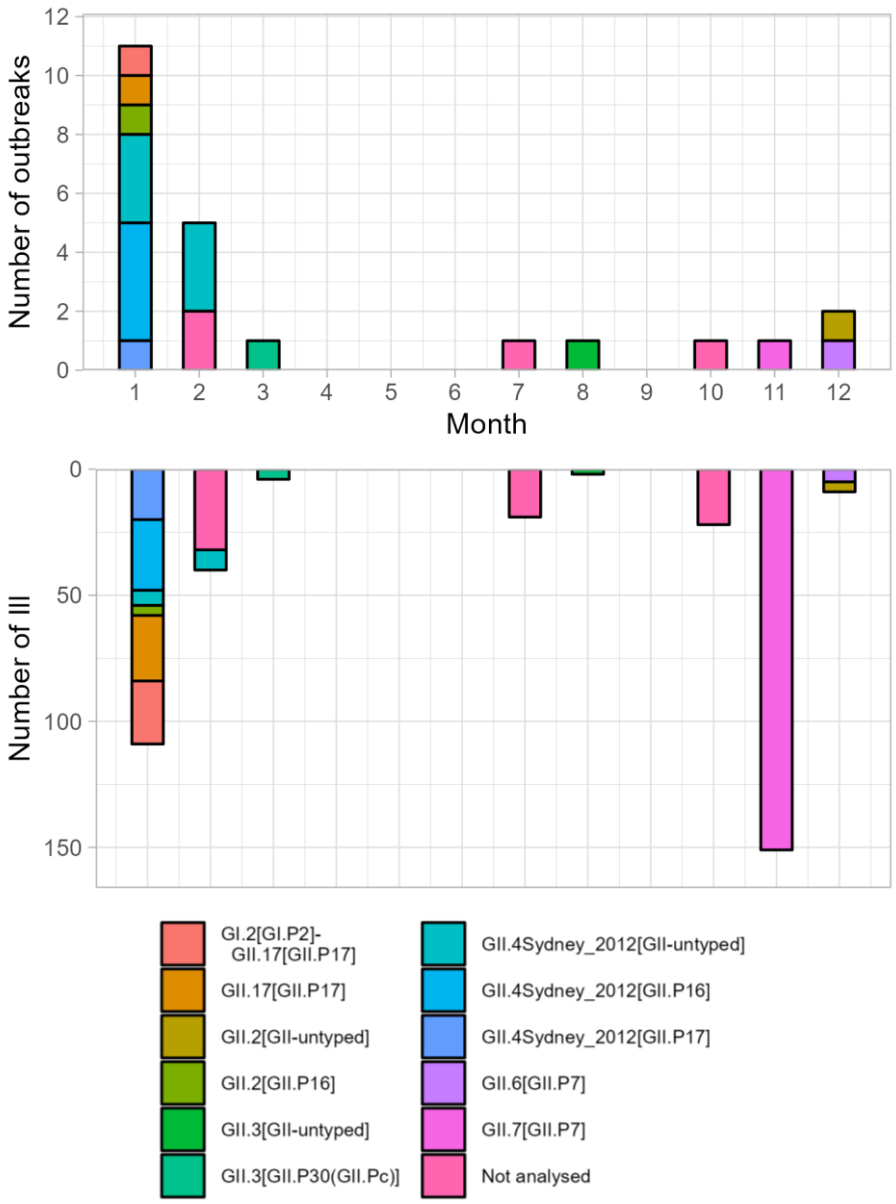

Figure 10. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2020. “Not analysed” comprises all samples that were either not received by the NRC, contained low levels of norovirus, or experienced unsuccessful sequencing.

As previously was shown that most norovirus GII.4 were associated with people older than 65 it can also be remarked that most of these infections occur in care facilities such as hospitals and nursing homes. Norovirus GII.P7, GII.P16 and GII.P17 could also be found in these settings. The genotypes more related to foodborne outbreaks were norovirus GII.3, GII.7 and GII.P7 (figure 11).

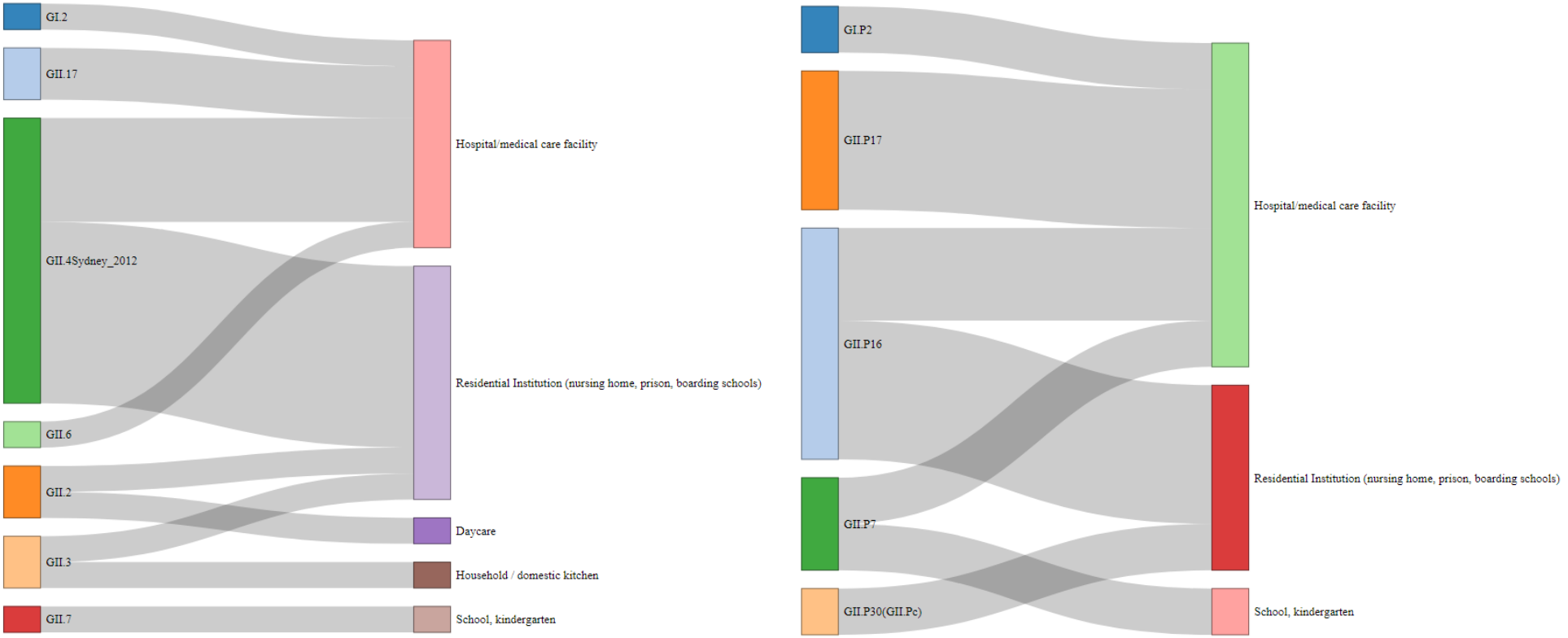

Figure 11. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the setting of the norovirus outbreaks in 2020.

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