NATIONAL REFERENCE CENTRE NOROVIRUS

Annual report 2019

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 2019, the National Reference Centre (NRC) received reports of 88 outbreaks potentially linked to Norovirus, affecting a minimum of 1468 individuals. Confirmation of Norovirus as the causative agent was established in 44 of these outbreaks, affecting a minimum of 997 individuals. Subsequent typing via sequencing allowed determination of Norovirus genogroup and genotype in 34 outbreaks, while technical limitations precluded such determination in the remaining 10 cases. Among the typed outbreaks, Norovirus genogroup GI was identified in 9 instances, while genogroup GII was predominant, detected in 30 outbreaks.

Of the reported outbreaks in 2019, 15 were suspected to involve Norovirus transmission through food. Norovirus was detected in human samples in 13 of these outbreaks Specifically, in 7 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 camps.

In 2019, the predominant circulating strain of Norovirus remained GII.4 (Sydney 2012) with 16 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 2019, the NRC Norovirus received a total of 389 human samples (see table 1). Diagnostic detection of human pathogenic Norovirus by reverse transcriptase (RT-)PCR was conducted on 353 of these samples, revealing the presence of the virus in 171 cases. Additionally, 36 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 2019

	Samples received
Total	389
Norovirus detected	207
Norovirus not detected	182
Outbreak	91
Sporadic	116

A total of 208 samples tested positive for norovirus. Among these, 91 were identified during an outbreak, while the remaining 116 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=11).

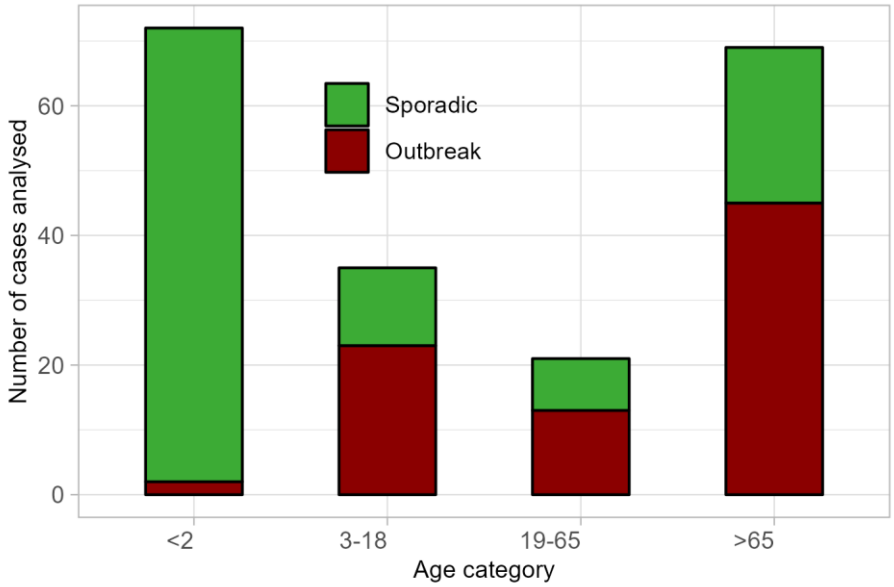

Figure 1. Norovirus detected in 2019 per age category.

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*., 2019).

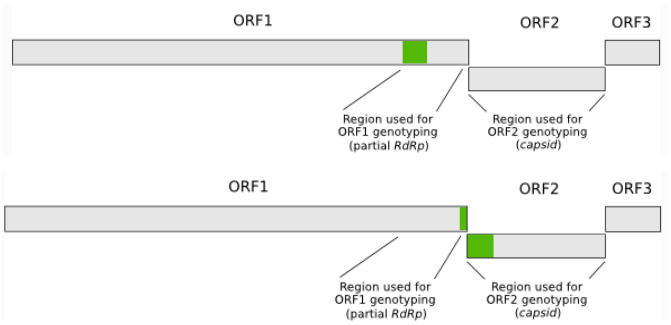

Figure 2. Schematic representation of the location of genomic regions used for genotyping of Norovirus (Vinjé et al 2004).

A total of 159 samples underwent genotyping, with the NRC successfully sequencing either the capsid region, the polymerase region, or both for 144 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 14 of the cases, 1 were detected by the clinical laboratory by RT-PCR.

Among the 159 samples, 50 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 7 of the samples contained the human pathogenic genogroup GI, 31 the human pathogenic genogroup GII and 4 both. The remaining 109 samples originated from sporadic cases. The serogrouping assay showed that 11 of the samples contained the human pathogenic genogroup GI, 88 the human pathogenic genogroup GII and 7 both.

Based on polymorphisms detected in the capsid gene, 7 different genotypes within genogroup GI (GI.3; GI.2; GI.1; GI.4; GI.6; GI.7) are distinguished in Belgium in 2019. A total of 10 different genotypes distinguished for genogroup GII (GII.4 Sydney 2012; GII.6; GII.2; GII.13; GII.3; GII.4; GII.17; GII.12; GII.14; GII.8). The following capsid types were detected for the first time by the NRC this year: poor quality, GII.13 and GII.8 .
Based on polymorphisms detected in the polymerase gene, 5 different P-types within gene group GI (GI.P3; GI.P2; GI.4; GI.P4; GI.P7) are distinguished in Belgium in 2019. A total of 10 different P-types distinguished for gene group GII (GII.P7; GII.P16; GII.P31; GII.P4; GII.P17; GII.P21; GII.P30; GII.P2; GII.P3; GII.P8). The following polymerase types were detected for the first time by the NRC this year: GII.P30 (former GII.Pc), GII.P3, GI.P2, GI.4, GII.P8. Figure 3. shows that multiple genotypes are associated with several P-types, especially within the genogroup GII.

Most of the norovirus GII.4 strains were detected in patients older than 65, whereas only a small portion of norovirus GII.3 cases were associated with this age category. The latter type was mainly found in children 3 to 18 of age. Remarkably, this year a significant 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, while P-type GII.P30 and GII.P31 were predominantly found in children under 2 years of age (see figure 4).

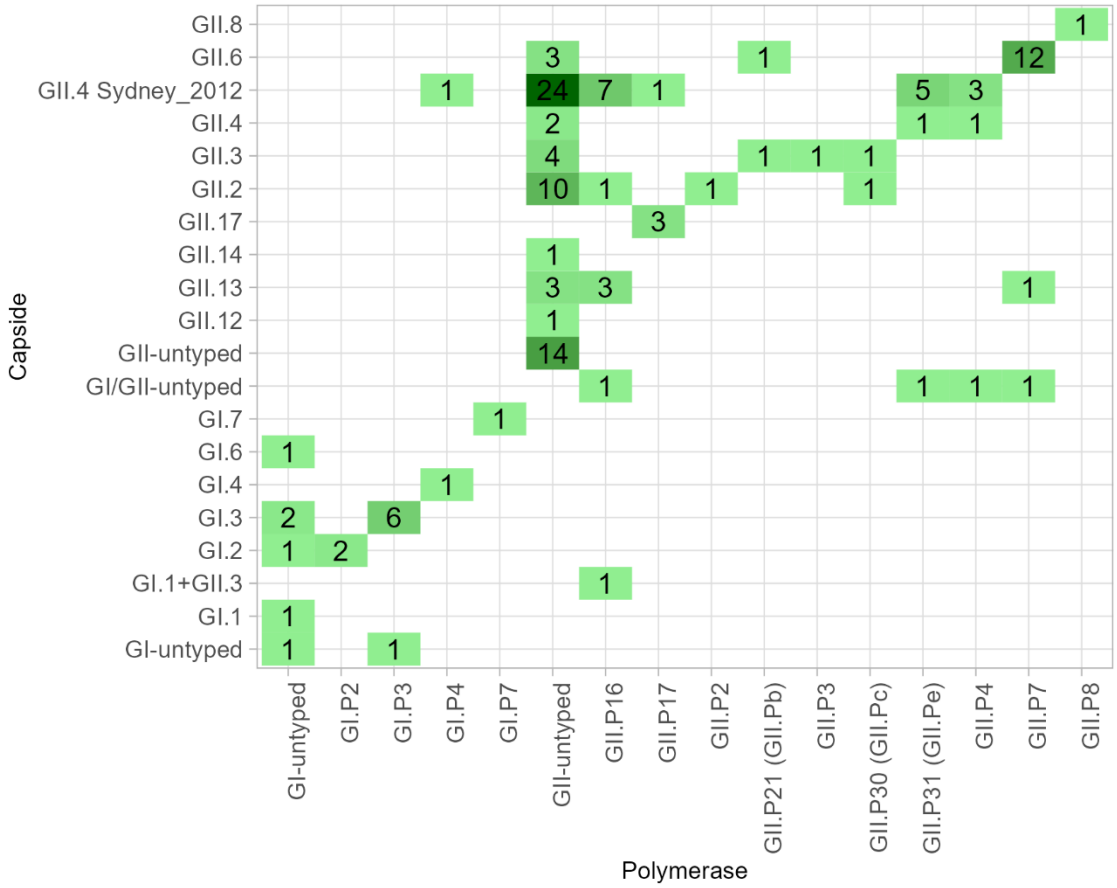

Figure 3. Norovirus capsid and polymerase dual type combinations

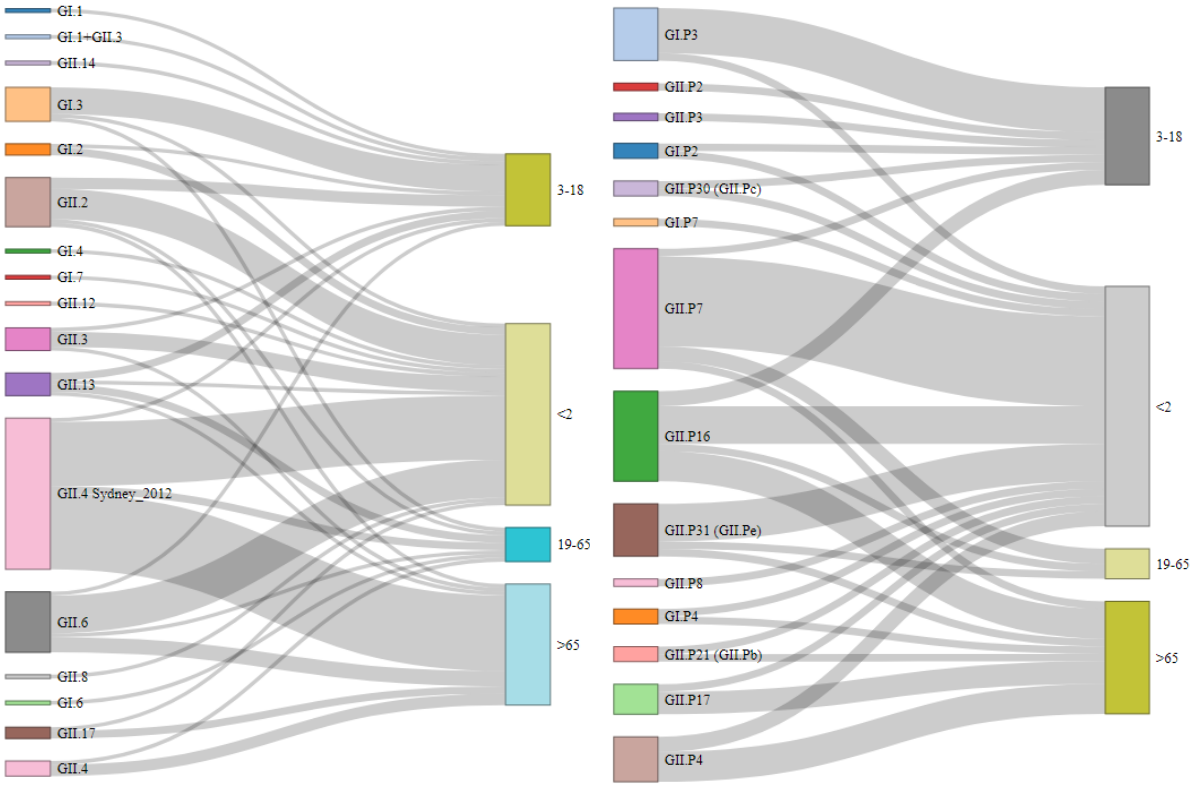

Figure 4. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the age category of the patient in 2019.

OUTBREAKS

1. General

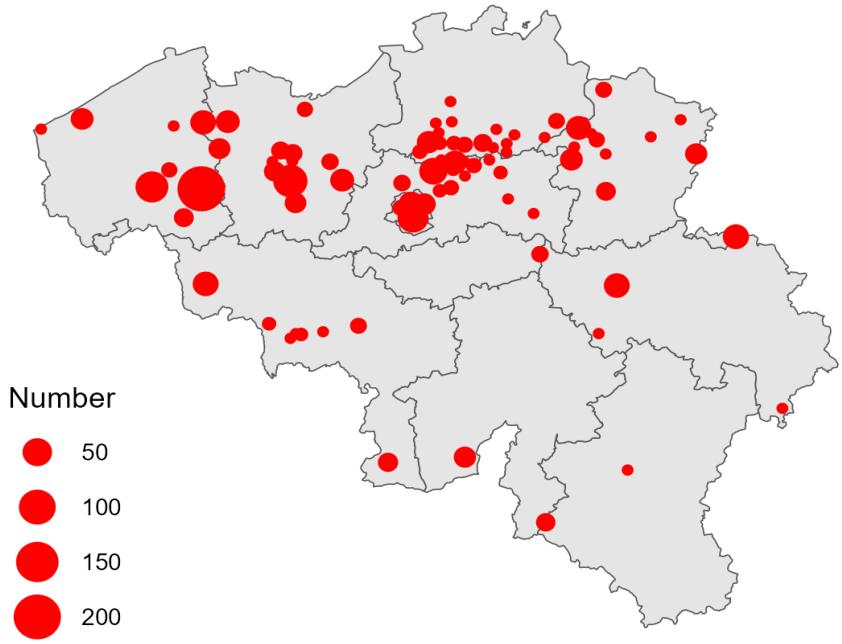

Figure 4. Geographical distribution of the norovirus outbreaks in 2019

The Service of foodborne pathogens of Sciensano houses both the NRC of Norovirus and the national reference laboratory (NRL) for foodborne outbreaks (FBO). In 2019 there were a total of 88 outbreak reports of acute gastroenteritis with a suspicion of norovirus infection. In 44 of these outbreaks norovirus was detected in human samples.

Investigations into 50 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 7 of the outbreaks norovirus was detected in the suspected food products. For 5 outbreaks, we were able to establish a clear link with the food product. In 8 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.

38 reported acute gastroenteritis outbreaks were not suspected to have arisen from foodborne transmission, with norovirus being detectable in 29 of these instances.

2. “… some more details”

In 2019, three distinct outbreaks were documented, each involving the detection of Norovirus in either food samples, human specimens from patients, or individuals involved in food preparation, suggesting a potential foodborne transmission route. The initial outbreak, occurring in Limburg, affected 26 individuals who exhibited symptoms such as vomiting, diarrhea, nausea, and fever within 48 hours post-consumption of sushi and oysters during a staff gathering. Analysis of the oyster left-overs revealed the presence of Norovirus GII, while unfortunately, no human samples were available for further investigation.

Subsequent outbreaks in West Flanders and Flemish Brabant, encompassing a total of 21 cases, displayed Norovirus solely within patient or food handler samples, with no evidence of contamination in the food itself. Despite the absence of viral presence in the food, the transmission was likely facilitated through a common food source. Specifically, the outbreak in West Flanders was attributed to GII.3[GII.P3] strain.

Throughout 2019, Norovirus predominantly contributed to outbreaks characterized by person-to-person transmission dynamics, Norovirus was implicated in several additional outbreaks, each deemed non-food-related. Notably, in a school outbreak, environmental swabs revealed the presence of Norovirus alongside reports of illness among individuals who had not consumed any food. Surprisingly, vomit samples yielded negative results for Norovirus. In contrast, the remaining outbreaks exhibited detectable Norovirus in human samples.

Throughout 2019, 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). However in 2019 this trend could not be observed in the NRC data, mainly due to the constant number of outbreak in every season of 2019.

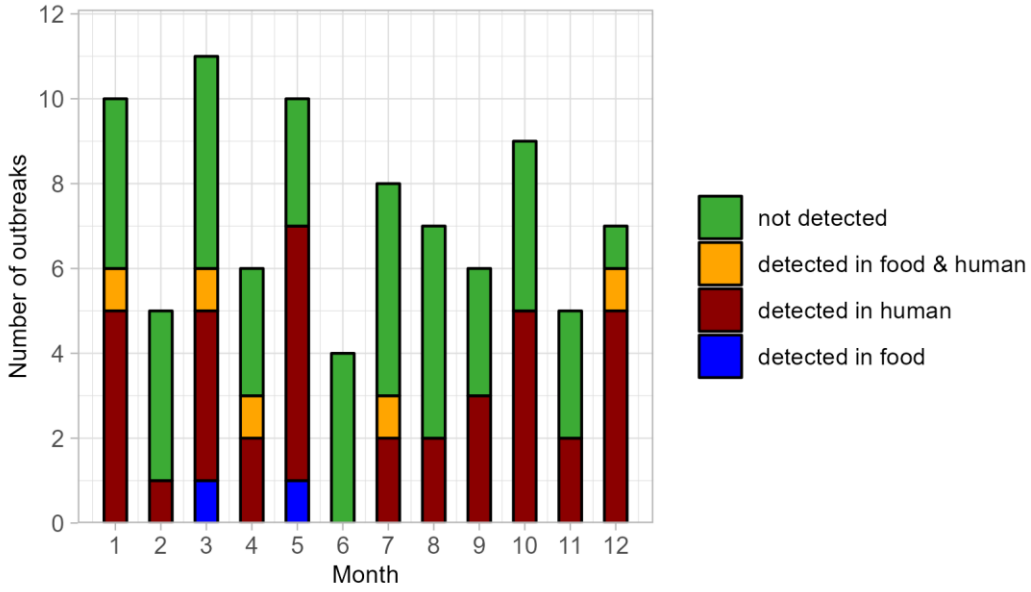

Figure 5. Number of acute gastroenteritis outbreaks reported to the NRC in 2019 per month.

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 25 of the acute gastroenteritis outbreaks.

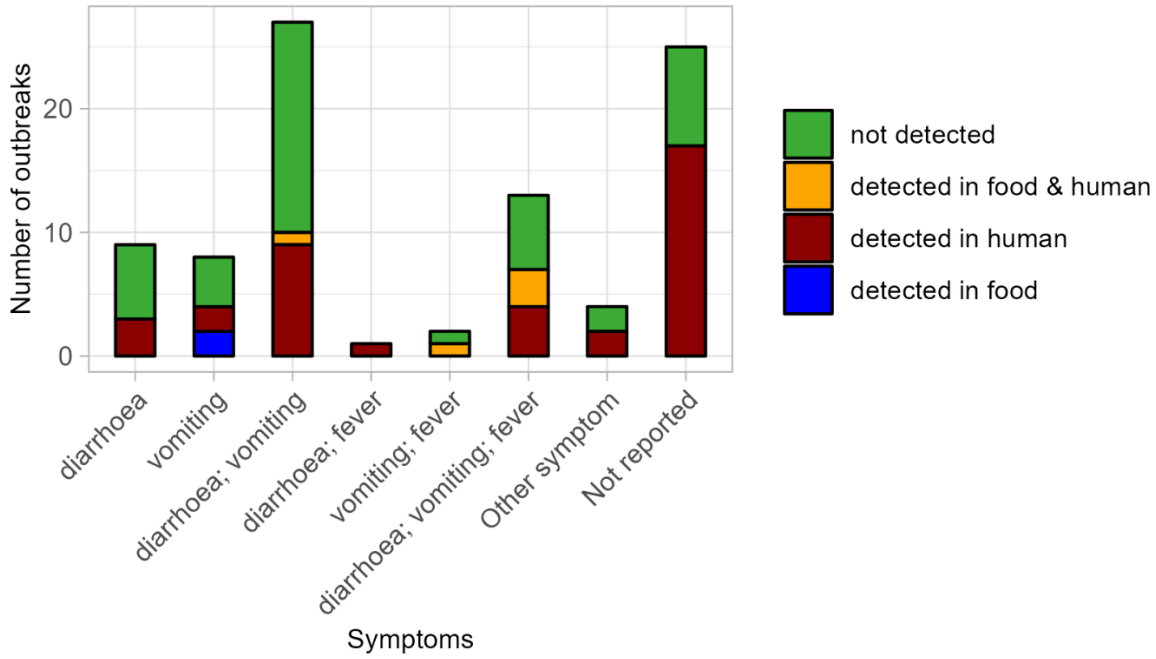

Figure 7. Reported symptoms associated with the AGE outbreaks reported to the NRC in 2019.

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 residential institutions and schools. Notably, the most substantial norovirus outbreak on record for 2019 was in a school encompassed 203 reported cases (figure 8).

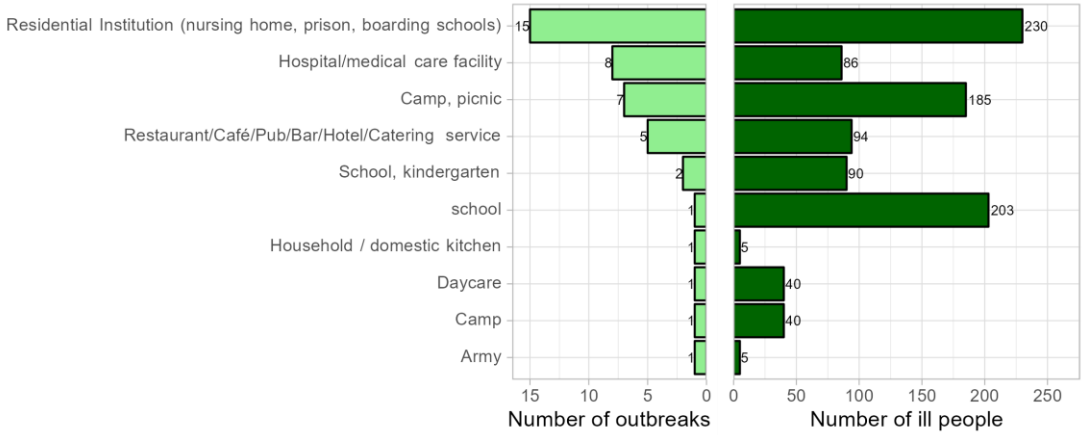

Figure 8. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2019.

5. Genotypes

The norovirus capsid polymorphism that was often detected in outbreaks in 2019 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, with its prevalence reaching its zenith in 2019, as it emerged 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.

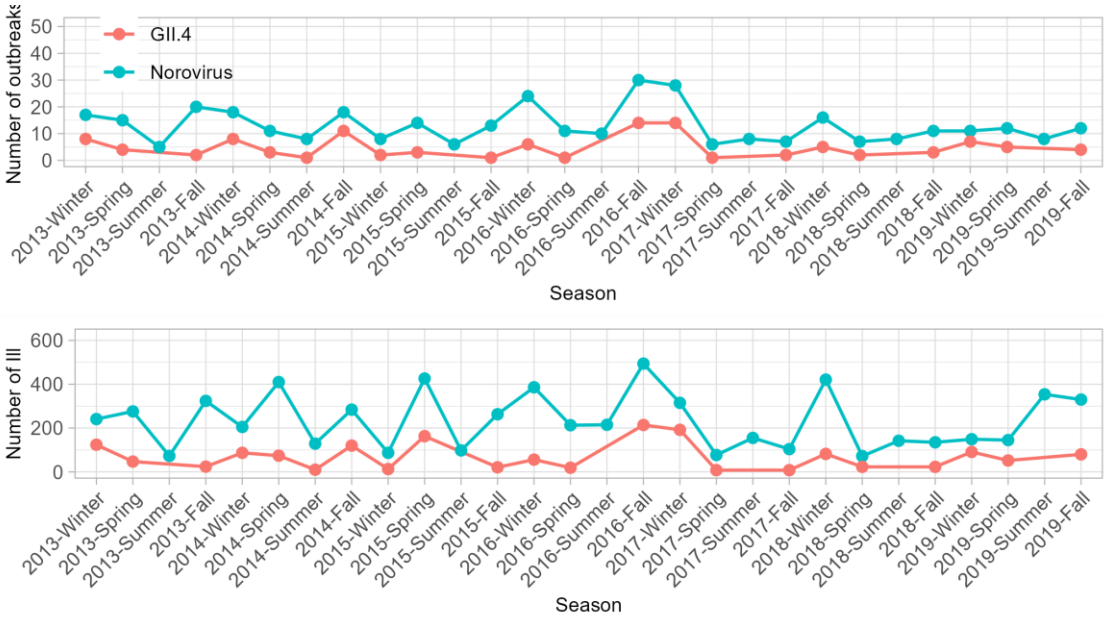

Figure 9. Number of norovirus and GII.4 outbreaks and Ill reported to the NRC since the start of the NRC activity.

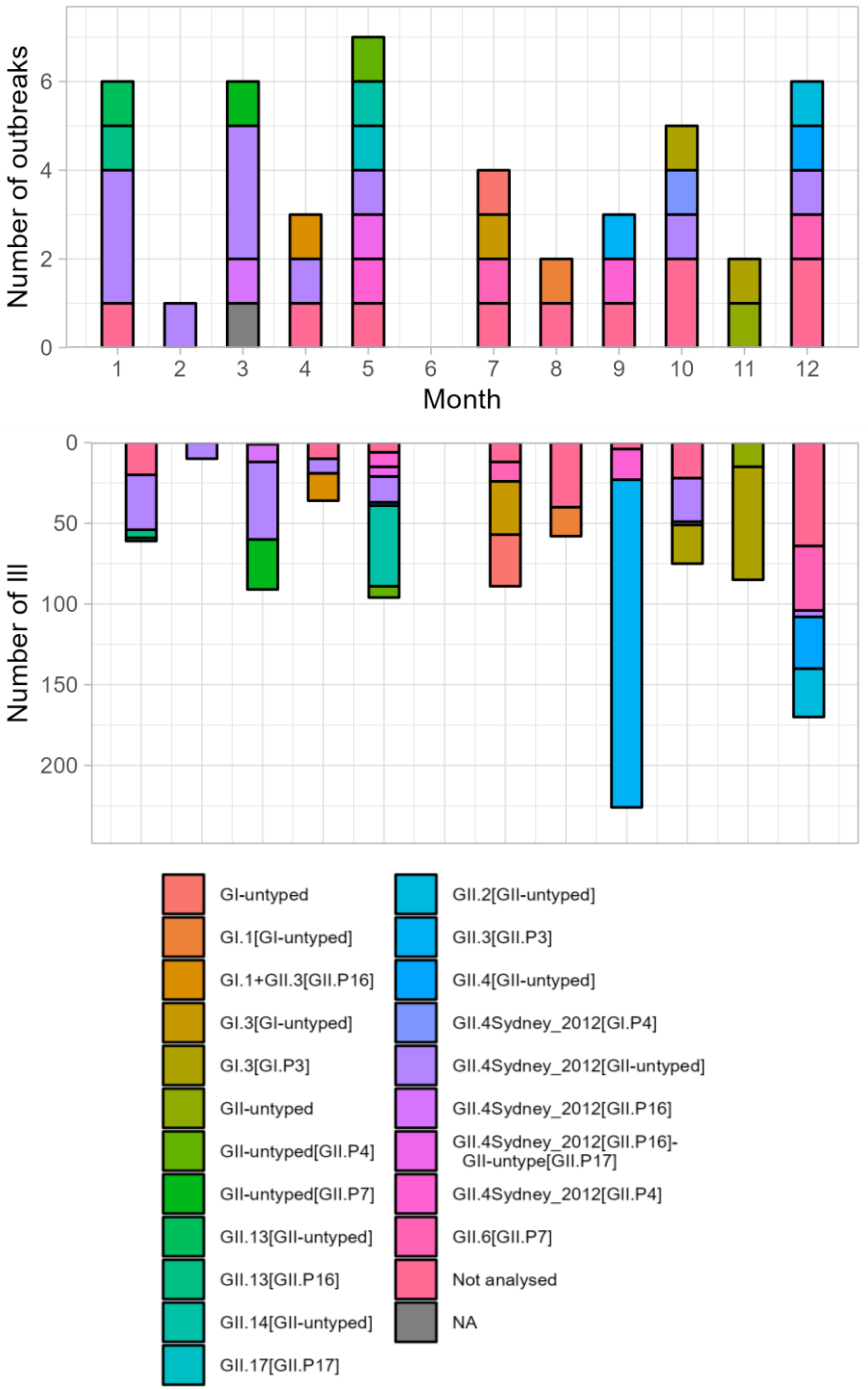

Figure 10. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2019. "Not analysed" comprises all samples that were either not received by the NRC, contained low levels of norovirus, or experienced unsuccessful sequencing.

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 GI.P4, GII.P4, GII.P16 and GII.P17 could also be found in these settings. The genotypes more related to foodborne outbreaks were norovirus GI.1, GI.3, GII.13, GII.3, GI.P3, GII.P3, GII.P16 and GII.P7 (figure 11).

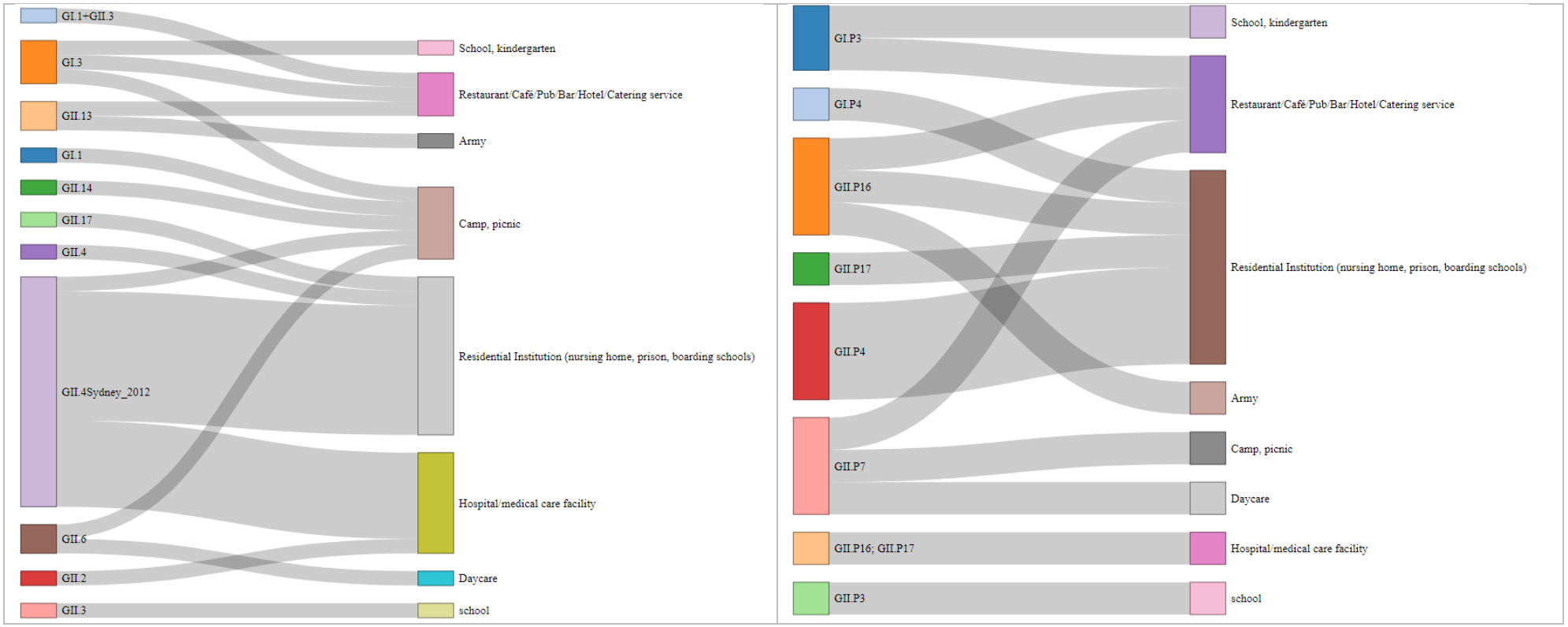

Figure 11. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the setting of the norovirus outbreaks in 2019.

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