NATIONAL REFERENCE CENTRE NOROVIRUS

Annual report 2018

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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acknowledgements

We express our thanks to the health inspectors who conduct patient surveys, as well as to the clinical laboratories, who, by sending their samples, cooperate in the surveillance of this pathogen. We also thank the Federal Agency for the Safety of the Food Chain (FASFC).

Please cite as: National Reference Centre for Norovirus, Annual report 2018. Sciensano, Brussels, Belgium

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EXECUTIVE SUMMARY

In 2018, the National Reference Centre (NRC) received reports of 72 outbreaks potentially linked to Norovirus, affecting a minimum of 1106 individuals. Confirmation of Norovirus as the causative agent was established in 41 of these outbreaks, affecting a minimum of 769 individuals. Subsequent typing via sequencing allowed determination of Norovirus genogroup and genotype in 27 outbreaks, while technical limitations precluded such determination in the remaining 14 cases. Among the typed outbreaks, Norovirus genogroup GI was identified in 17 instances, while genogroup GII was predominant, detected in 22 outbreaks.

Of the reported outbreaks in 2018, 11 were suspected to involve Norovirus transmission through food. Norovirus was detected in human samples from all of these outbreaks Specifically, in 4 outbreaks, the transmission via food could be confirmed. Notably, the majority of Norovirus reports originated from hospitals, medical care facility and residential institution such as nursing home, prison, boarding schools.

In 2018, the predominant circulating strain of Norovirus remained GII.4 (Sydney 2012) with 8 outbreaks, initially identified in September 2012 by van Beek et al. Additionally, Norovirus genotype GI.3 was implicated in 4 outbreaks during this period.

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OVERVIEW OF ACTIVITIES

1. Norovirus detection

In 2018, the NRC Norovirus received a total of 512 human samples (see table 1). Diagnostic detection of human pathogenic Norovirus by reverse transcriptase (RT-)PCR was conducted on 422 of these samples, revealing the presence of the virus in 176 cases. Additionally, 90 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 2018

	Samples received
Total	512
Norovirus detected	266
Norovirus not detected	246
Outbreak	95
Sporadic	171

A total of 266 samples tested positive for norovirus. Among these, 95 were identified during an outbreak, while the remaining 171 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=15).

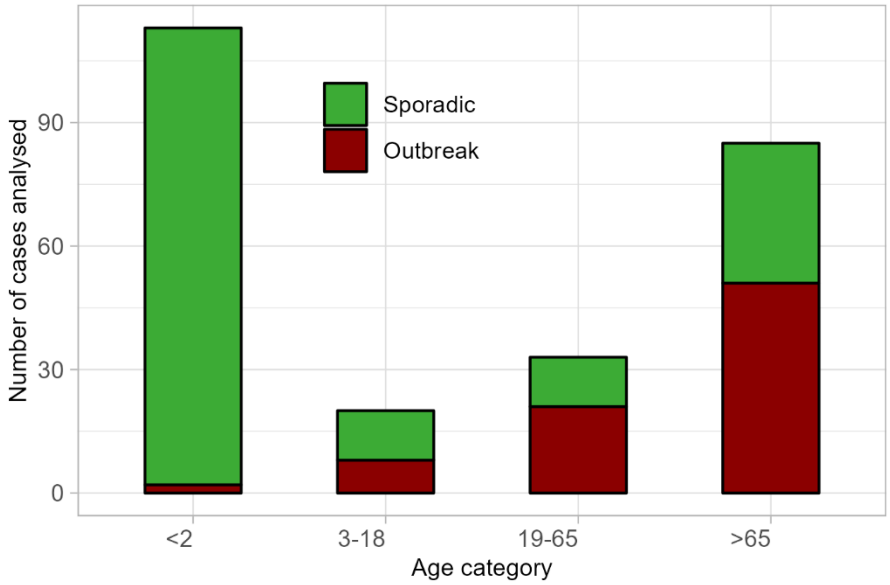

Figure 1. Norovirus detected in 2018 per age category.

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.

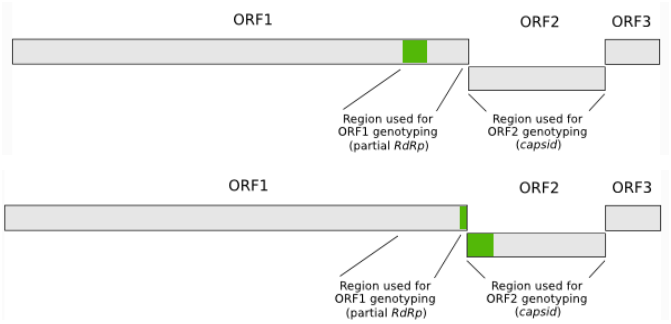

Figure 2. Schematic representation of the location of genomic regions used for genotyping of Norovirus (Vinjé et al 2004).

A total of 171 samples underwent genotyping, with the NRC successfully sequencing either the capsid region, the polymerase region, or both for 139 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 23 of the cases, 9 were detected by the clinical laboratory by RT-PCR.

Among the 171 samples, 52 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 8 of the samples contained the human pathogenic genogroup GI, 26 the human pathogenic genogroup GII and 7 both. The remaining 119 samples originated from sporadic cases. The serogrouping assay showed that 4 of the samples contained the human pathogenic genogroup GI, 80 the human pathogenic genogroup GII and 12 both.

Based on polymorphisms detected in the capsid gene, 3 different genotypes within genogroup GI (GI.3; GI.4; GI.1) are distinguished in Belgium in 2018. A total of 9 different genotypes distinguished for genogroup GII (GII.4 Sydney 2012; GII.3; GII.17; GII.7; GII.2; GII.4; GII.6; GII.12; GII.4 Osaka 2007). The following capsid types were detected for the first time by the NRC this year: GI.1, GII.4 Osaka 2007 .
Based on polymorphisms detected in the polymerase gene, 3 different P-types within gene group GI (GI.P4; GI.Pb; GI.Pd) are distinguished in Belgium in 2018. A total of 10 different P-types distinguished for gene group GII (GII.P21; GII.P4; GII.P16; GII.P17; GII.P4 New Orleans 2009; GII.P31; GII.P7; GII.P12; GII.P21; GII.P2). The following polymerase types were detected for the first time by the NRC this year: GII.P12, GI.Pb, GI.Pd . 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 under 2 years of age. All polymerase types associated with GII.4 were detected in patients older than 65, and no particular P-type was predominantly found in children under 2 years of age (see figure 4).

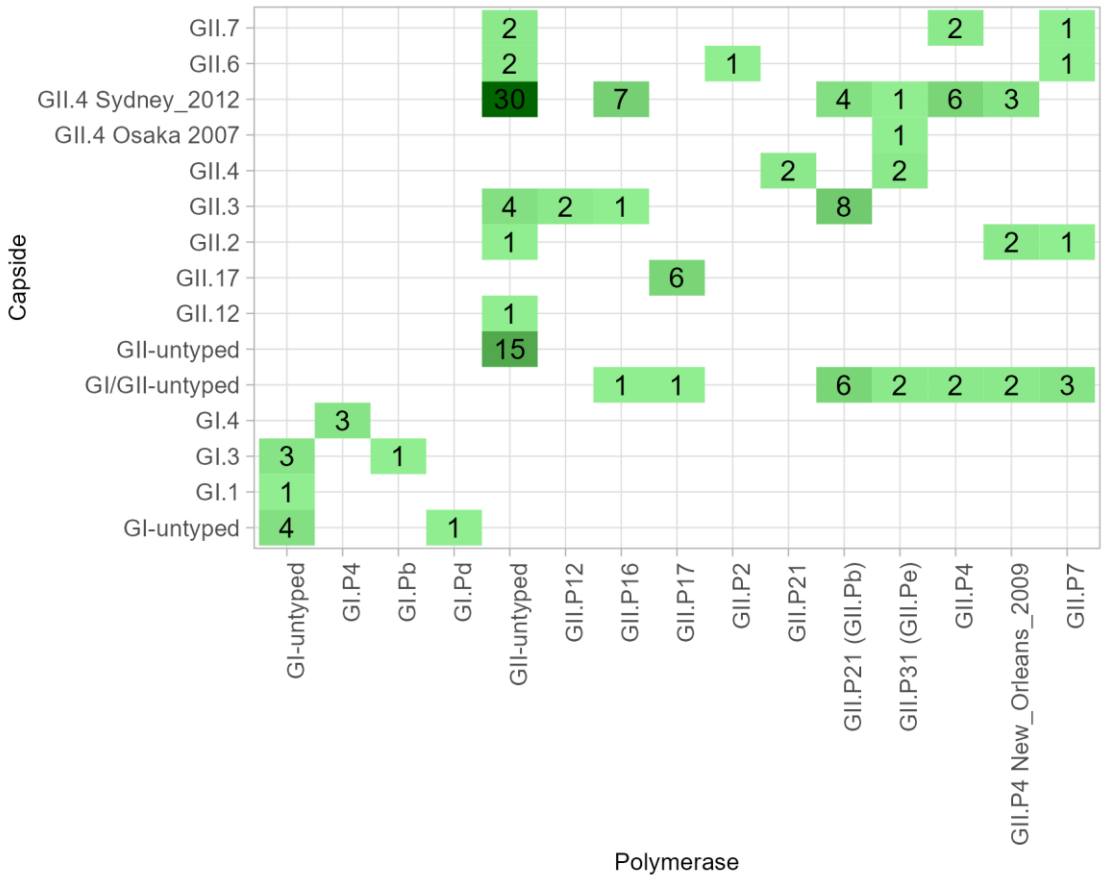

Figure 3. Norovirus capsid and polymerase dual type combinations

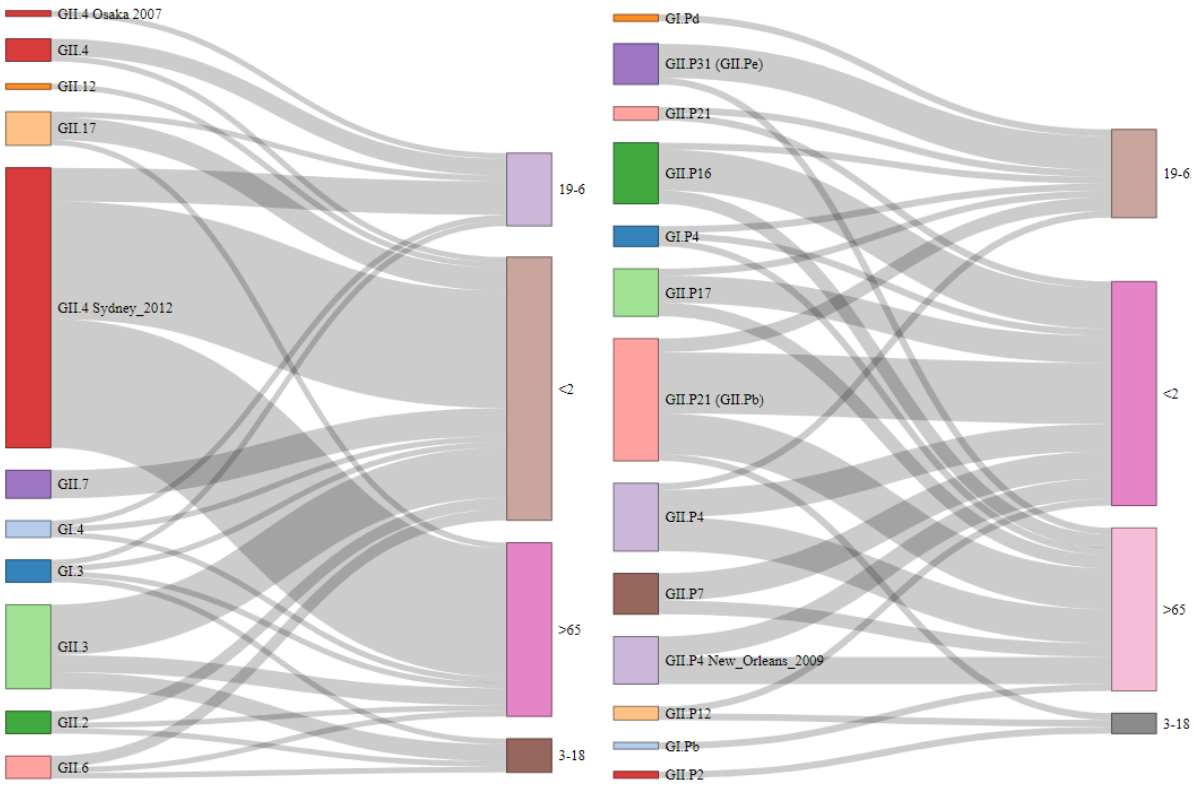

Figure 4. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the age category of the patient in 2018

OUTBREAKS

1. General

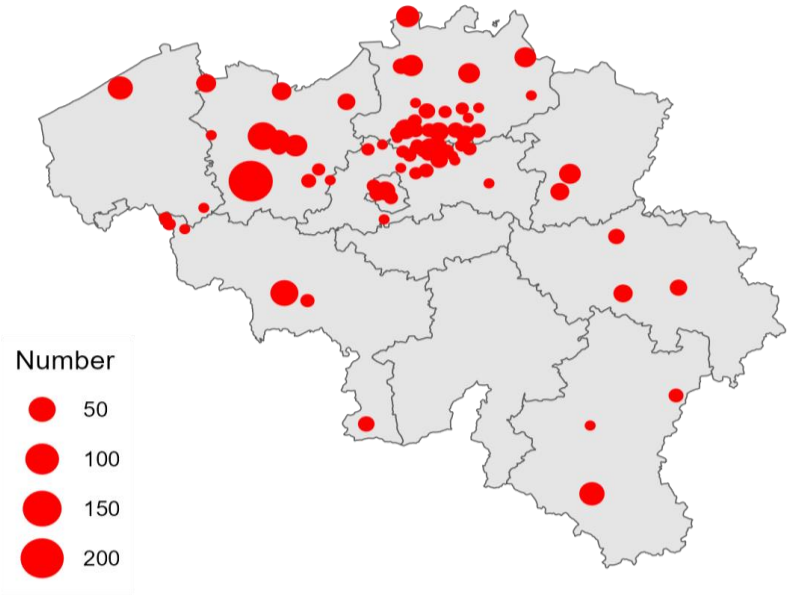

Figure 4. Geographical distribution of the norovirus outbreaks in 2018

The Service of foodborne pathogens of Sciensano houses both the NRC of Norovirus and the national reference laboratory (NRL) for foodborne outbreaks (FBO). In 2018 there were a total of 72 outbreak reports of acute gastroenteritis with a suspicion of norovirus infection. In 41 of these outbreaks norovirus was detected in human samples. Based on the investigation in 36 of these cases food was suspected to be involved in the transmission of Norovirus 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 4 of the outbreaks norovirus was detected in the suspected food products and a clear link with the food product could be established. In 7 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.

36 reported acute gastroenteritis outbreaks were not suspected to have arisen from foodborne transmission, with norovirus being detectable in 30 of these instances.

2. “… some more details”

Throughout 2018, four separate outbreaks were documented with transmission via contaminated food. In three of these incidents, Norovirus genotype GII was. The initial outbreak involved four individuals who presented with symptoms of vomiting, diarrhea, and fever subsequent to consuming a meal at a restaurant, with Norovirus GII identified in both patients and the restaurant proprietor.

The second outbreak occurred within various establishments affiliated with the same school group in East Flanders, resulting in illness among 212 children. The predominant symptoms observed among the affected individuals were vomiting. These sites received supplies from a central wholesale kitchen, which had prepared a total of 800 meals. Notably, a food preparer responsible for handling raw vegetables and sandwich preparation also exhibited symptoms indicative of infection. Epidemiological investigation, including the construction of an epidemiological curve, suggested a common point source of contamination, followed by secondary transmission via person-to-person contact. Both human samples (vomit and stool) and a retained sample[1] tested positive for Norovirus GII, confirming the foodborne transmission. Further typing of the virus in human samples revealed the presence of GII.3 [GII.P12] strain.

Another Norovirus outbreak in 2018, associated with the detection of Norovirus GI.3 among the affected individuals, failed to yield positive results for the virus in food samples. Nonetheless, the epidemiological evidence suggested a potential link between the outbreak and food consumption. Specifically, seven children fell ill during a scout camp, presenting with symptoms of vomiting and diarrhea.

Throughout 2018, 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.

[1] retained sample refers to a portion of a product that is retained by the manufacturer, distributor, or importer for future reference or analysis. This sample is kept in case there are quality control issues, regulatory inquiries, or customer complaints arise.

Figure 5 illustrates the typical seasonal dynamics of norovirus, showcasing a prominent surge in outbreak occurrences during the fall-winter months (November-March). As observed in 2017, in 2018 an additional peak was evident in July.

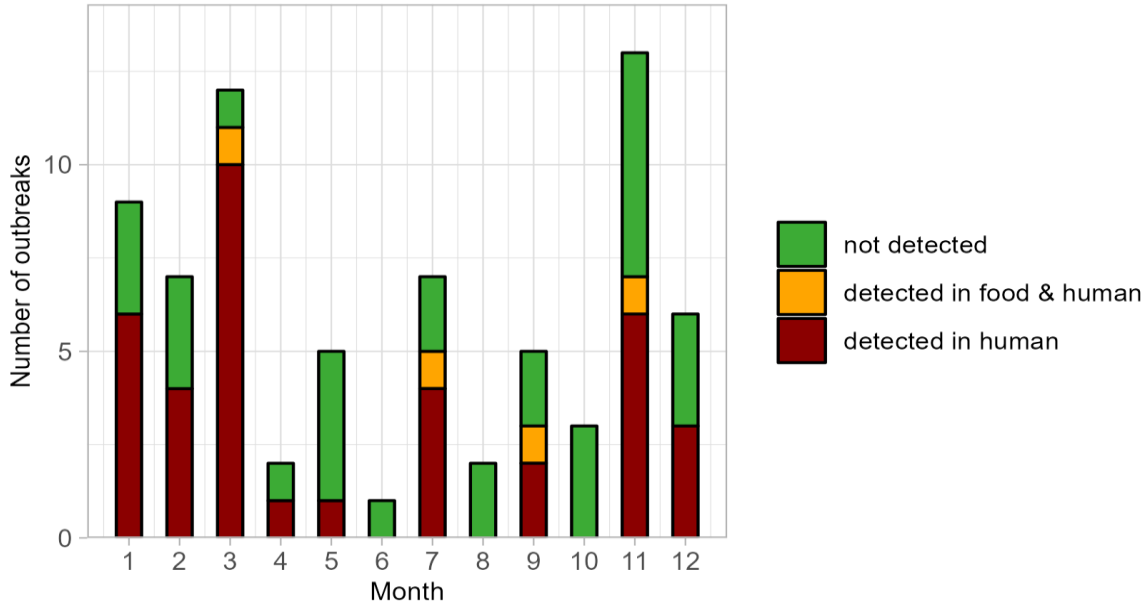

Figure 5. Number of acute gastroenteritis outbreaks reported to the NRC in 2018 per month.

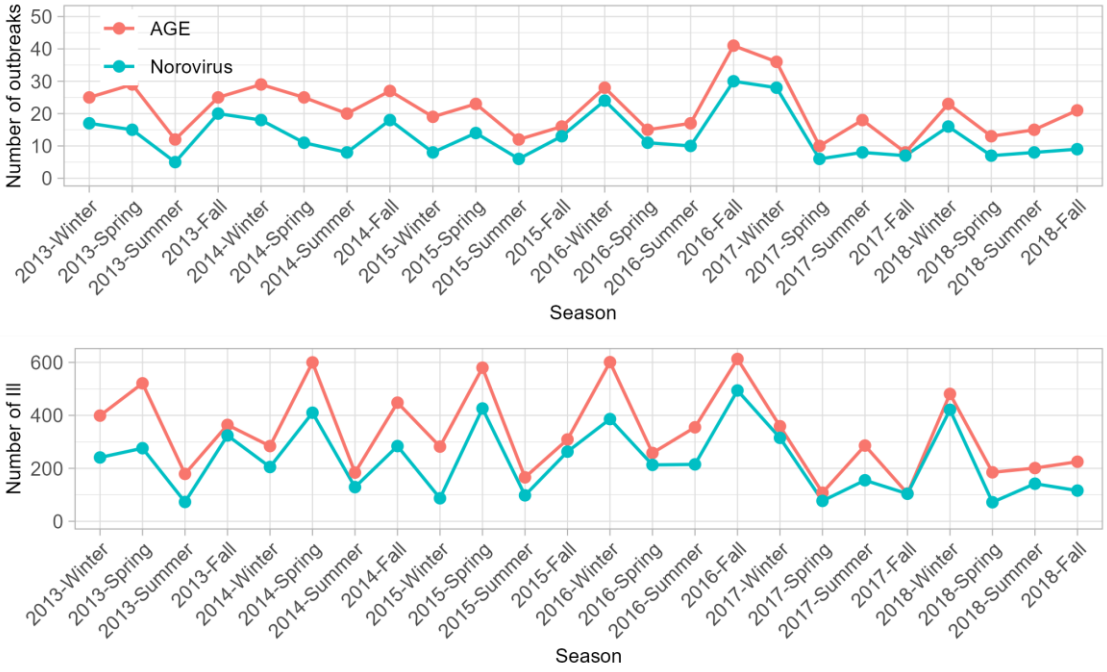

Figure 6. Number of acute gastroenteritis (AGE) and norovirus outbreaks (top) and number of ill (bottom) reported to the NRC since 2013.

3. Symptoms

Most norovirus outbreaks were associated with diarrhoea; vomiting cases. The symptoms of the affected cases were not reported for 18 of the acute gastroenteritis outbreaks.

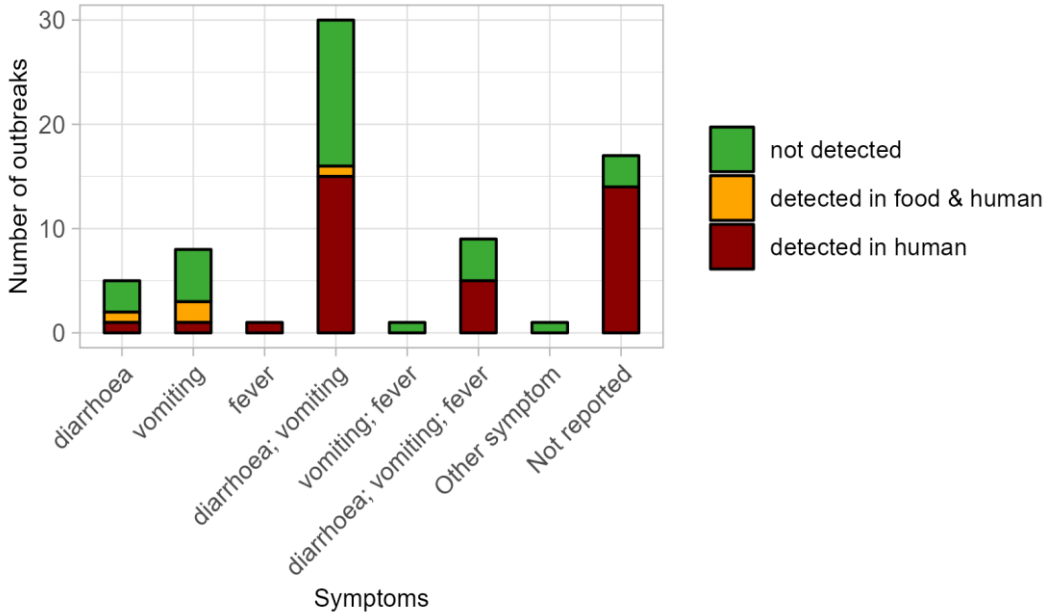

Figure 7. Reported symptoms associated with the AGE outbreaks reported to the NRC in 2018.

4. Setting

Norovirus outbreaks predominantly occurred in several key settings: hospitals and medical care facilities, residential institutions, and camps. However, the outbreaks with the most reported cases took place in schools and kindergartens. Notably, the most substantial norovirus outbreak on record for 2018 was in schools encompassed 212 reported cases (figure 8).

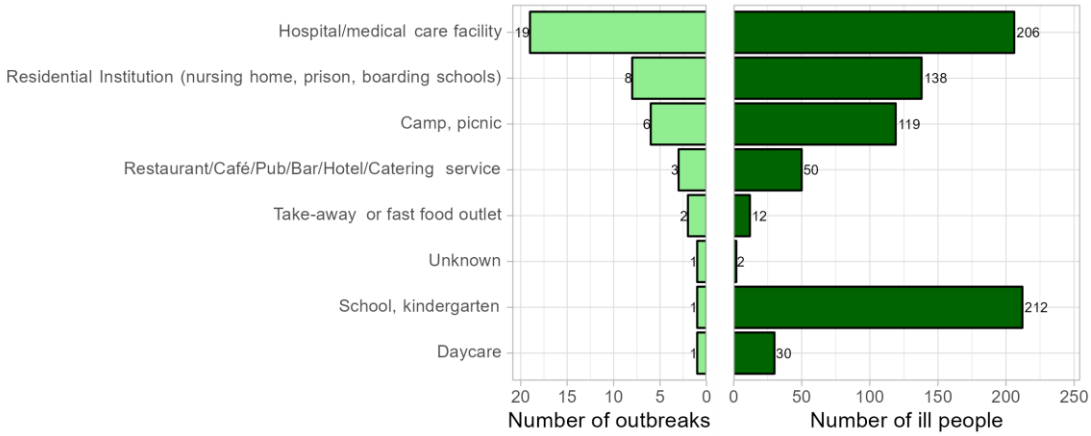

Figure 8. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2018.

5. Genotypes

The norovirus capsid polymorphism that was often detected in outbreaks in 2018 was GII.4 Sydney 2012 and the most often detected polymerase was GII.P21(former GII.Pb) (figure 10). 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. Notably, peaks are observed during winter and norovirus GII.4 emerges as the predominant strain during inter-peak periods.

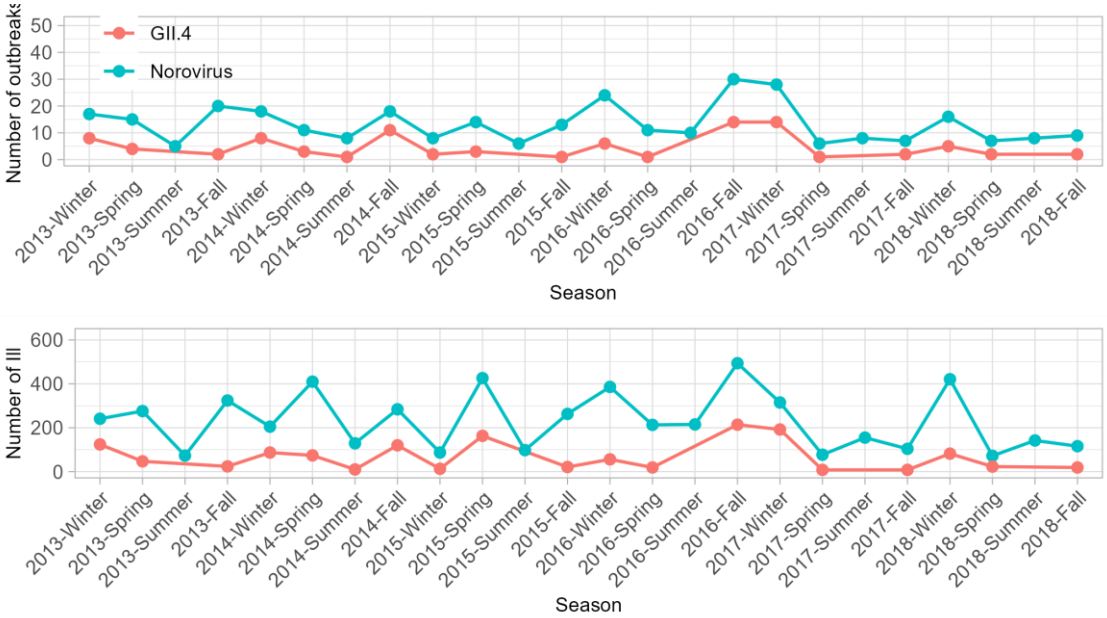

Figure 9. Number of norovirus and GII.4 outbreaks and Ill reported to the NRC since the start of the NRC activity.

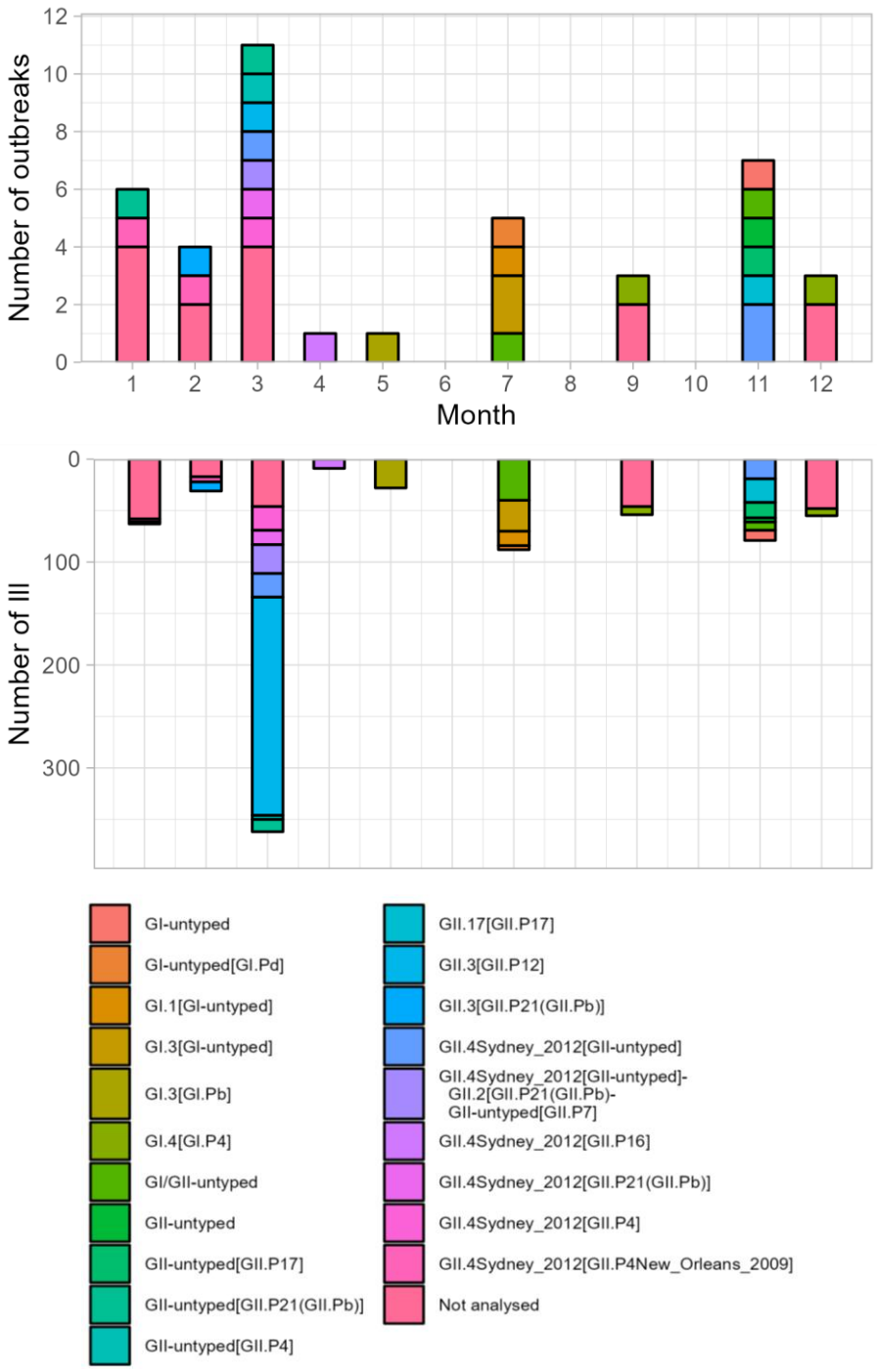

Figure 10. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2018.

"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.P7, GII.P16, GII.P17 and GII.P21 could also be found in these settings. The genotypes more related to foodborne outbreaks were norovirus GI.1, GI.3, GI.4, GII.3, GI.P4, GII.P12 and GI.Pd (figure 11).

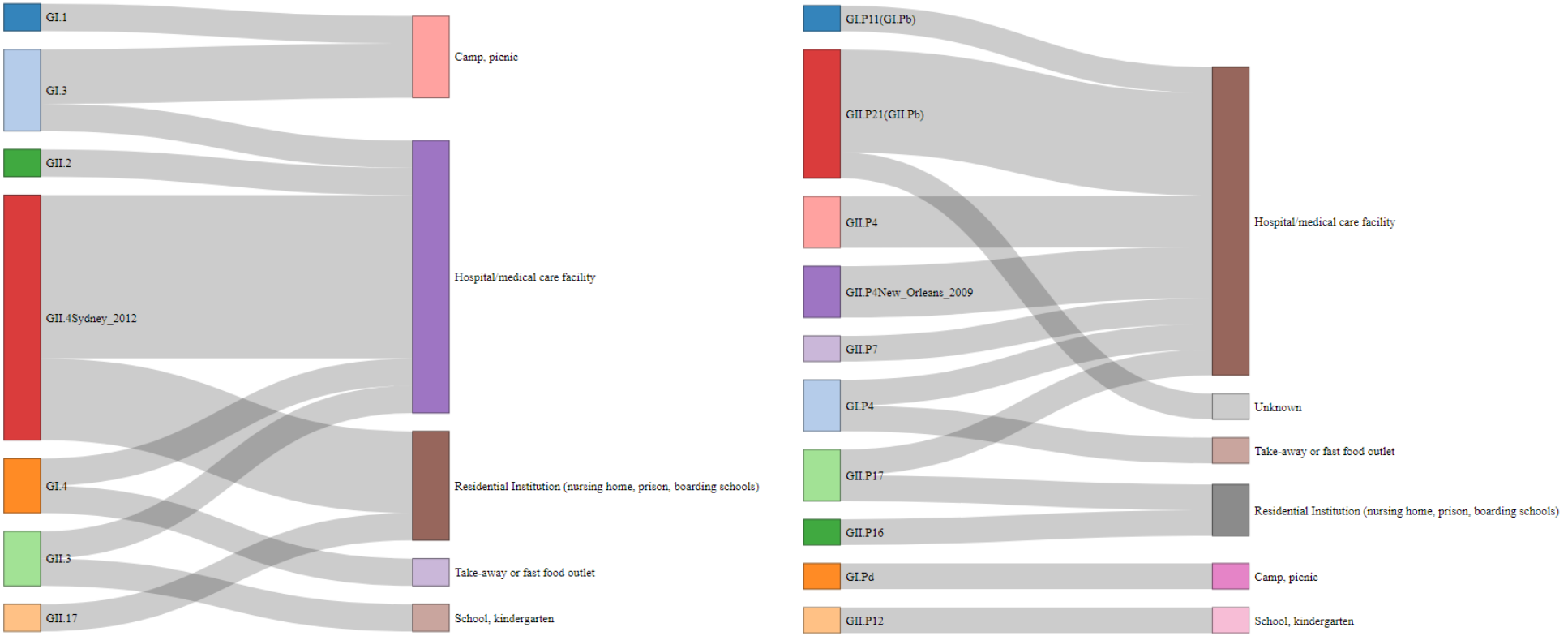

Figure 11. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the setting of the norovirus outbreaks in 2018.

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- Voedselvergiftigingen in België, jaarverslag 2018, Sciensano

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