NATIONAL REFERENCE CENTRE NOROVIRUS

Annual report 2017

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

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EXECUTIVE SUMMARY

In 2017, the National Reference Centre (NRC) received reports of 75 outbreaks potentially linked to Norovirus, affecting a minimum of 902 individuals. Confirmation of Norovirus as the causative agent was established in 52 of these outbreaks, affecting a minimum of 695 individuals. Subsequent typing via sequencing allowed determination of Norovirus genogroup and genotype in 30 outbreaks, while technical limitations precluded such determination in the remaining 22 cases. Among the typed outbreaks, Norovirus genogroup GI was identified in 5 instances, while genogroup GII was predominant, detected in 30 outbreaks. The genogroup could not be ascertained in 0 outbreaks.

Of the reported outbreaks in 2017, 8 were suspected to involve Norovirus transmission through food. Norovirus was detected in human samples from all of these outbreaks. Specifically, in 2 outbreaks, the transmission was linked to food. Notably, the majority of Norovirus reports originated from hospitals, medical care facility and residential Institution such as nursing home, prison, boarding schools.

In 2017, the predominant circulating strain of Norovirus remained GII.4 (Sydney 2012) with 17 outbreaks, initially identified in September 2012 by van Beek et al. Additionally, Norovirus genotype GII.2 was implicated in 3 outbreaks during this period.

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OVERVIEW OF ACTIVITIES

1. Norovirus detection

In 2017, the NRC Norovirus received a total of 355 human samples (see table 1). Diagnostic detection of human pathogenic Norovirus by reverse transcriptase (RT-)PCR was conducted on 298 of these samples, revealing the presence of the virus in 204 cases. Additionally, 57 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 2017

	Samples received
Total	355
Norovirus detected	261
Norovirus not detected	94
Outbreak	147
Sporadic	114

A total of 262 samples tested positive for norovirus. Among these, 147 were identified during an outbreak, while the remaining 114 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=20).

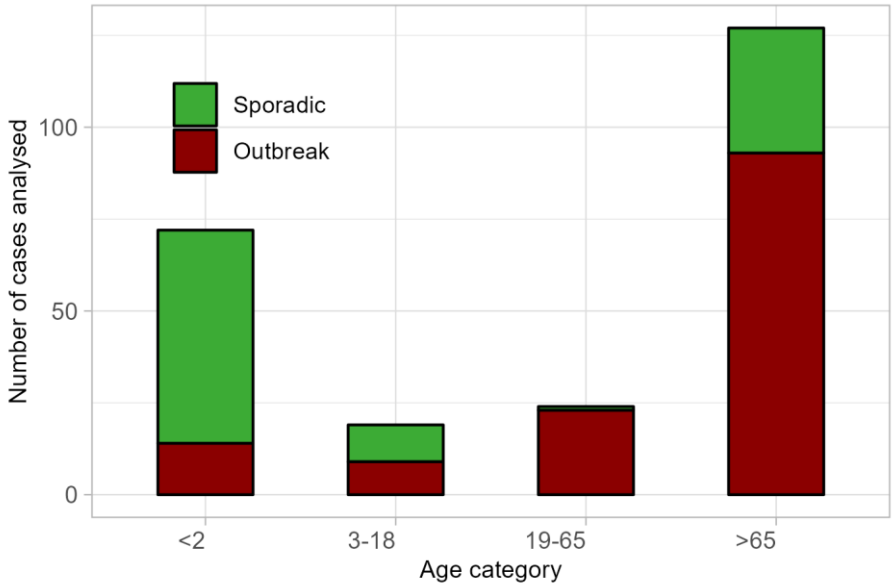

Figure 1. Norovirus detected in 2017 per age category.

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.

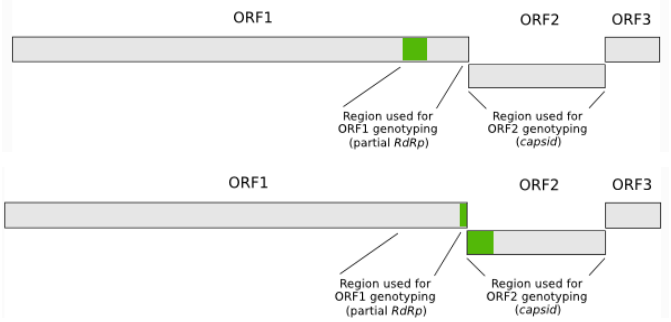

Figure 2. Schematic representation of the location of genomic regions used for genotyping of Norovirus (Vinjé et al 2004).

A total of 181 samples underwent genotyping, with the NRC successfully sequencing either the capsid region, the polymerase region, or both for 114 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 18 of the cases, 49 were detected by the clinical laboratory by RT-PCR.

Among the 181 samples, 77 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 4 of the samples contained the human pathogenic genogroup GI, 42 the human pathogenic genogroup GII and 7 both.
The remaining 104 samples originated from sporadic cases. The serogrouping assay showed that 3 of the samples contained the human pathogenic genogroup GI, 45 the human pathogenic genogroup GII and 13 both.

Based on polymorphisms detected in the capsid gene, 2 different genotypes within genogroup GI (GI.7; GI.3) are distinguished in Belgium in 2017. A total of 8 different genotypes distinguished for genogroup GII (GII.4 Sydney 2012; GII.3; GII.2; GII.6; GII.13; GII.4; GII.12; GII.7). The following capsid types were detected for the first time by the NRC this year: GII.13. Based on polymorphisms detected in the polymerase gene, 3 different P-types within gene group GI (GI.P7; GI.P4; GI.P3) are distinguished in Belgium in 2017. A total of 7 different P-types distinguished for gene group GII (GII.P21; GII.P4; GII.P16; GII.P4 New Orleans 2009; GII.P7; GII.P3; GII.P2). The following polymerase types were detected for the first time by the NRC this year: GI.P7. 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).

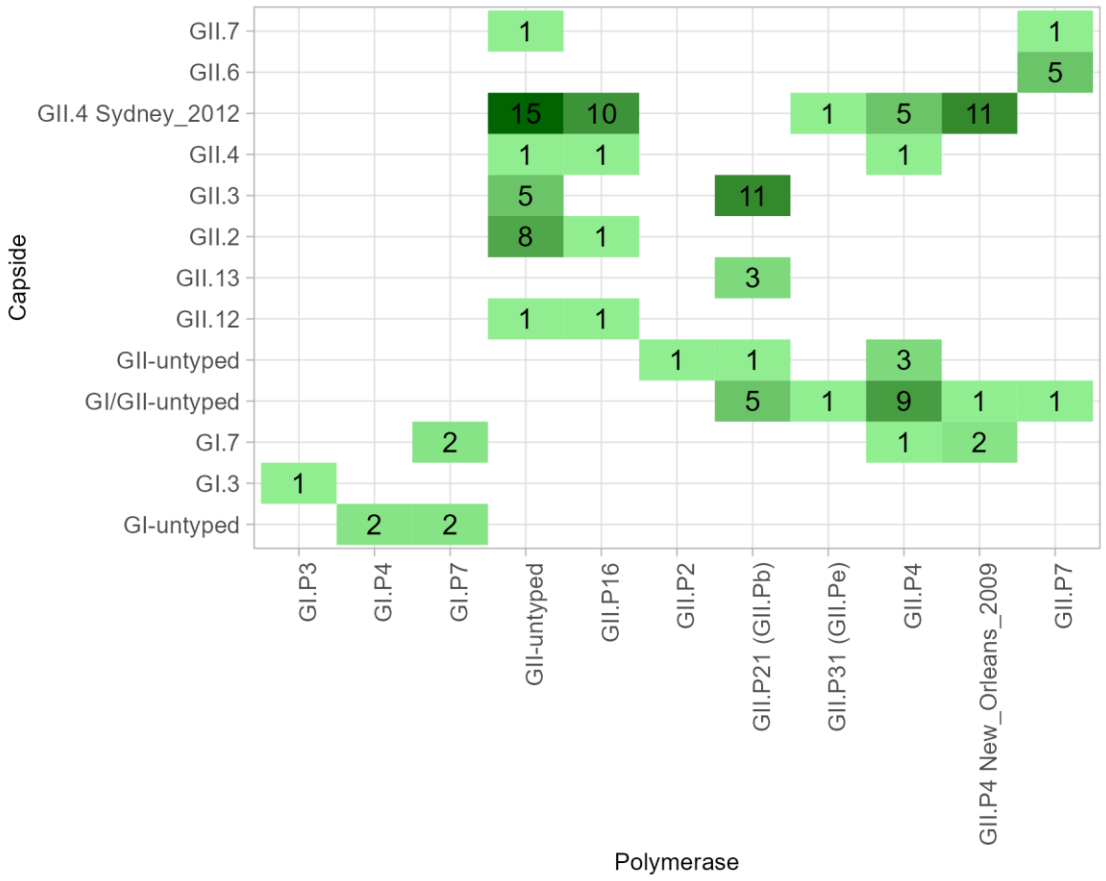

Figure 3. Norovirus capsid and polymerase dual type combinations

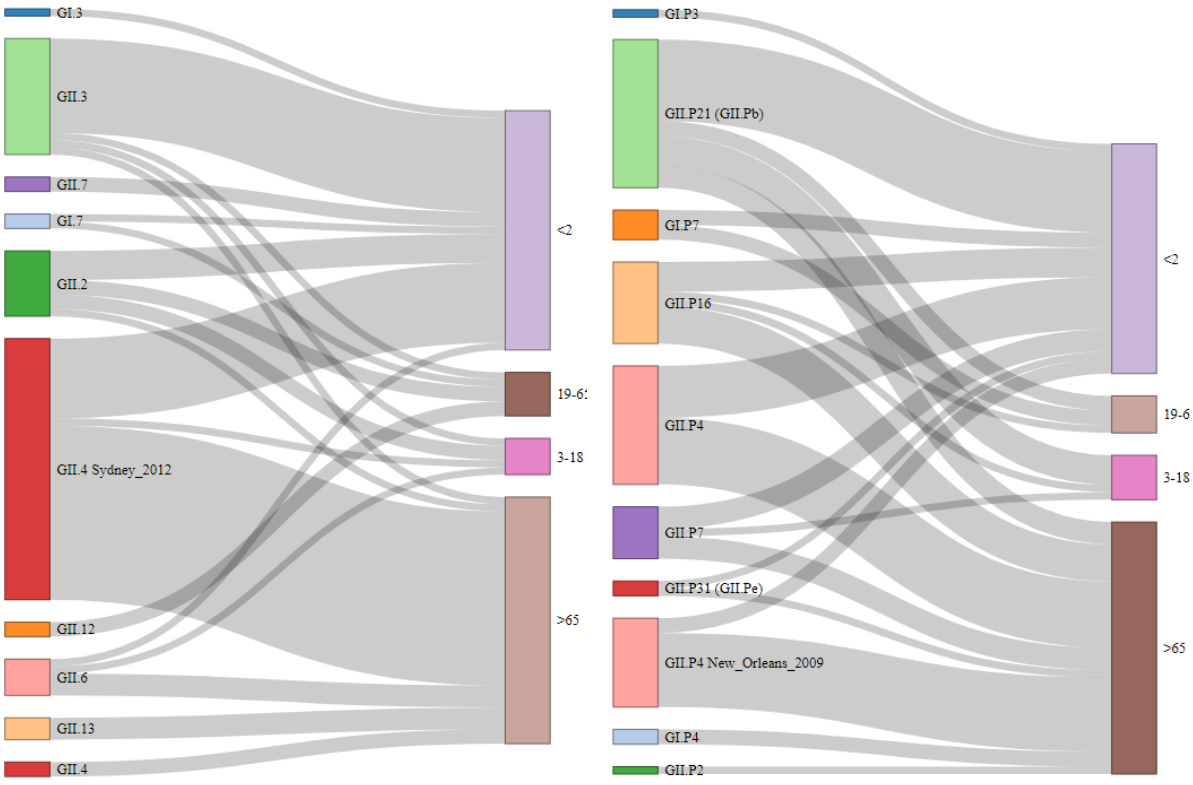

Figure 4. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the age category of the patient in 2017

OUTBREAKS

1. General

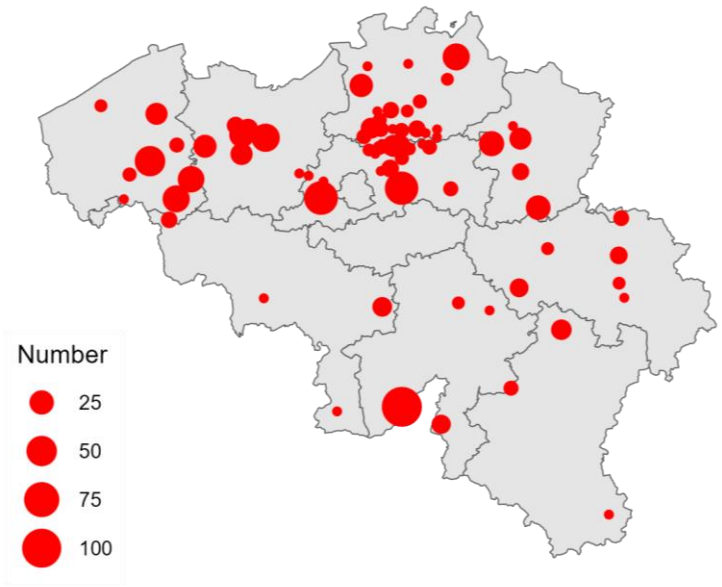

Figure 4. Geographical distribution of the norovirus outbreaks in 2017

The Service of foodborne pathogens of Sciensano houses both the NRC of Norovirus and the national reference laboratory (NRL) for foodborne outbreaks (FBO). In 2017 there were a total of 75 outbreak reports of acute gastroenteritis with a suspicion of norovirus infection.

In 52 of these outbreaks norovirus was detected in human samples. Based on the investigation in 21 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 2 of the outbreaks norovirus was detected in the suspected food products and a clear link with the food product established. In 6 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.

The remaining 54 reported acute gastroenteritis outbreaks were not suspected to have a foodborne transmission and norovirus could be detected in 44 of the outbreaks.

2. “… some more details”

Throughout 2017, three outbreaks were reported, wherein Norovirus was identified in samples obtained from affected individuals or food handlers, suggesting foodborne transmission. Of these, two outbreaks evidenced the presence of Norovirus genotype GI in both human cases and implicated food items. The first outbreak involved 50 individuals who exhibited symptoms of vomiting, diarrhea, and fever subsequent to consuming a stone-grilled meal featuring raw ingredients. Notably, Norovirus GI.7 was detected in both the afflicted individuals and the food handlers, along with their close contacts. Furthermore, eight food samples tested positive for Norovirus GI, underscoring foodborne transmission. Environmental samples, however, yielded no evidence of Norovirus.

The second outbreak transpired among a group of schoolchildren during a retreat, wherein several children (n=10) fell ill, four of whom required hospitalization. Investigation revealed that a food preparer, who had displayed symptoms of vomiting and diarrhea the preceding night, had served at the breakfast table. Subsequent testing of leftover sandwiches and human stool samples from the afflicted individuals confirmed the presence of Norovirus GI.

Conversely, in one of the 2017 outbreaks, Norovirus could not be detected in food samples; nevertheless, foodborne transmission was presumed. A total of 30 individuals fell ill following a buffet-style meal, exhibiting symptoms of vomiting and diarrhea, with Norovirus detected in human samples.

Throughout 2017, 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 winter months. Notably, in 2017, an additional peak was evident in July.

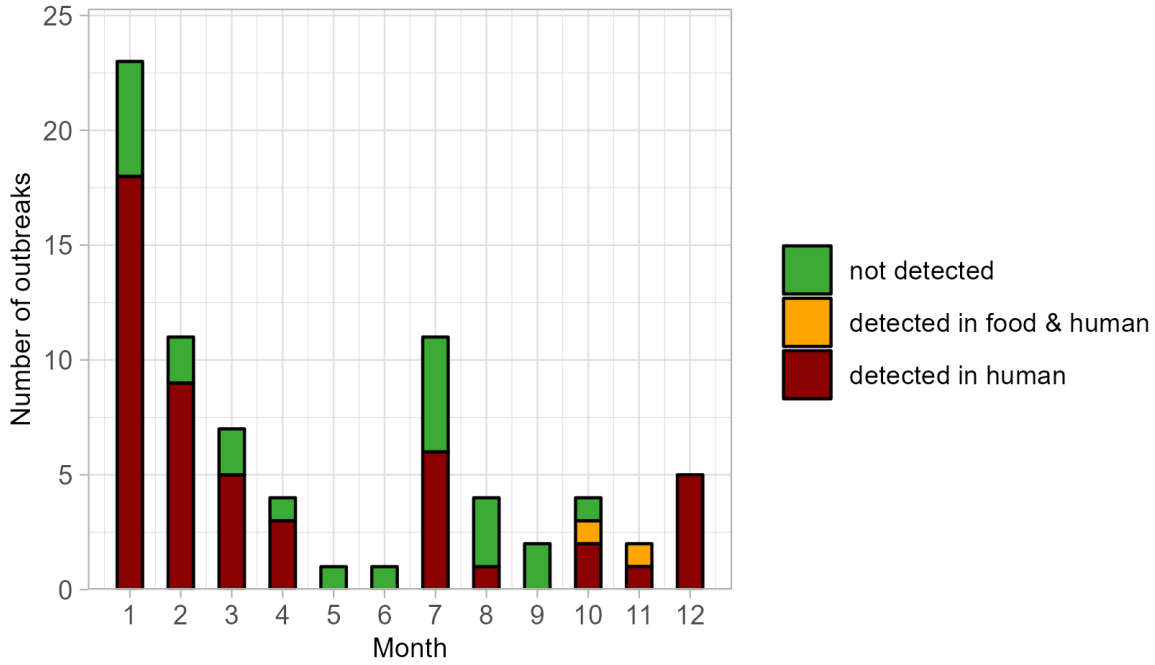

Figure 5. Number of acute gastroenteritis outbreaks reported to the NRC in 2017 per month.

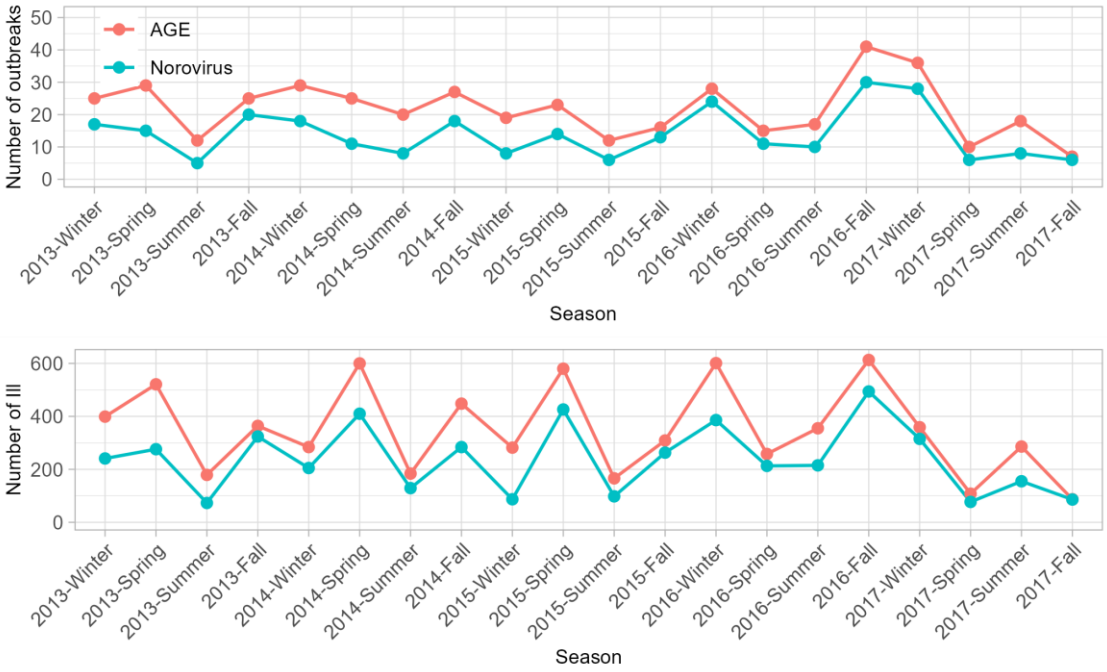

Figure 6. Number of acute gastroenteritis (AGE) and norovirus outbreaks (top) and number of ill (bottom) reported to the NRC since 2013.

3. Symptoms

Most norovirus outbreaks were associated with diarrhoea; vomiting cases. The symptoms of the affected cases were not reported for 19 of the acute gastroenteritis outbreaks.

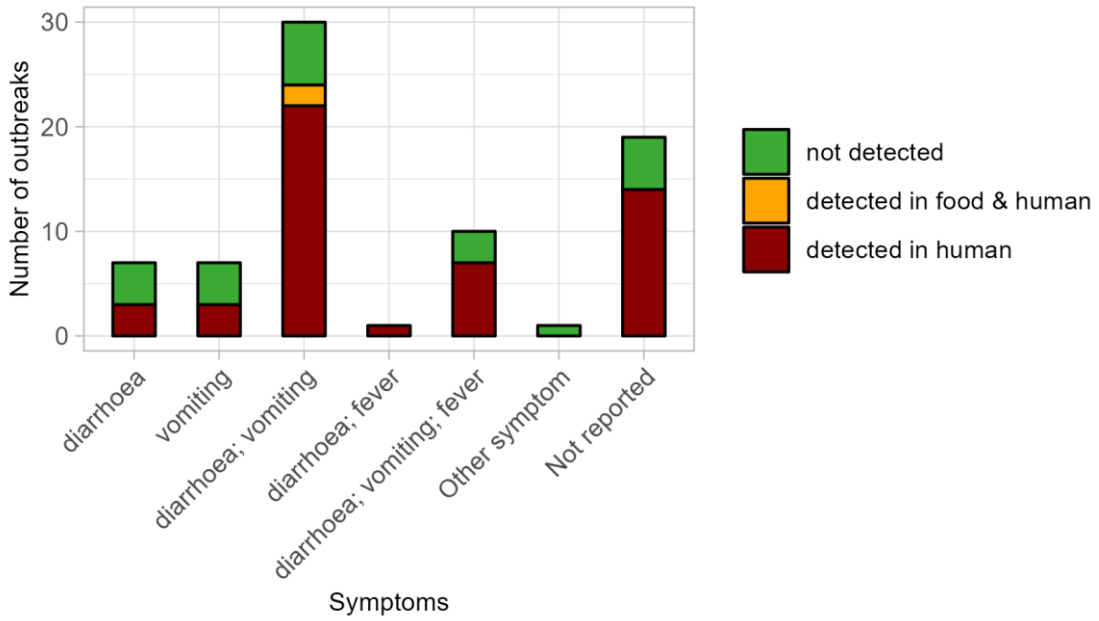

Figure 7. Reported symptoms associated with the AGE outbreaks reported to the NRC in 2017.

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 the residential institutions. Notably, the most substantial norovirus outbreak on record within a residential institution for 2017 encompassed 55 reported cases (figure 8).

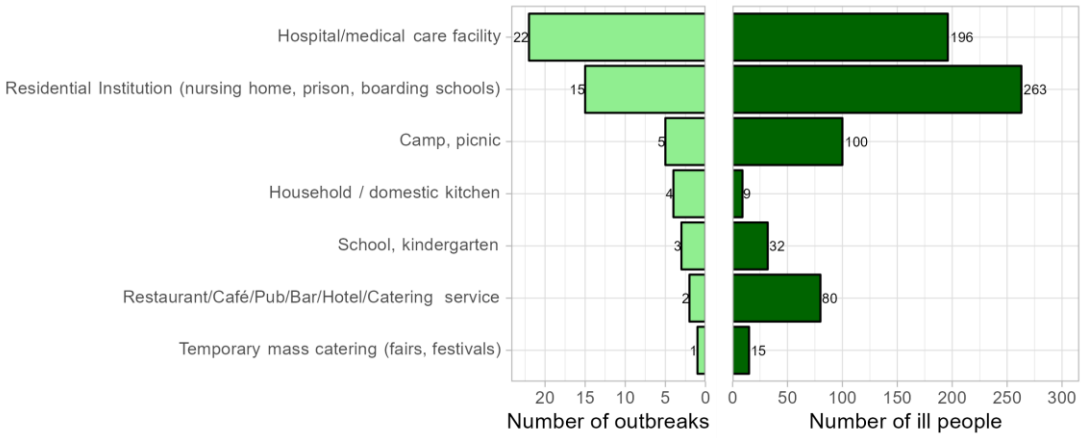

Figure 8. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2017.

5. Genotypes

The norovirus capsid polymorphism that was often detected in outbreaks in 2017 was GII.4 Sydney 2012 and the most often detected polymerase was GII.P4 New Orleans 2009 (figure 10). Consequently the norovirus genotype that was often detected in outbreaks in 2017 was GII.4 Sydney 2012[GII.P4 New Orleans 2009].

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.

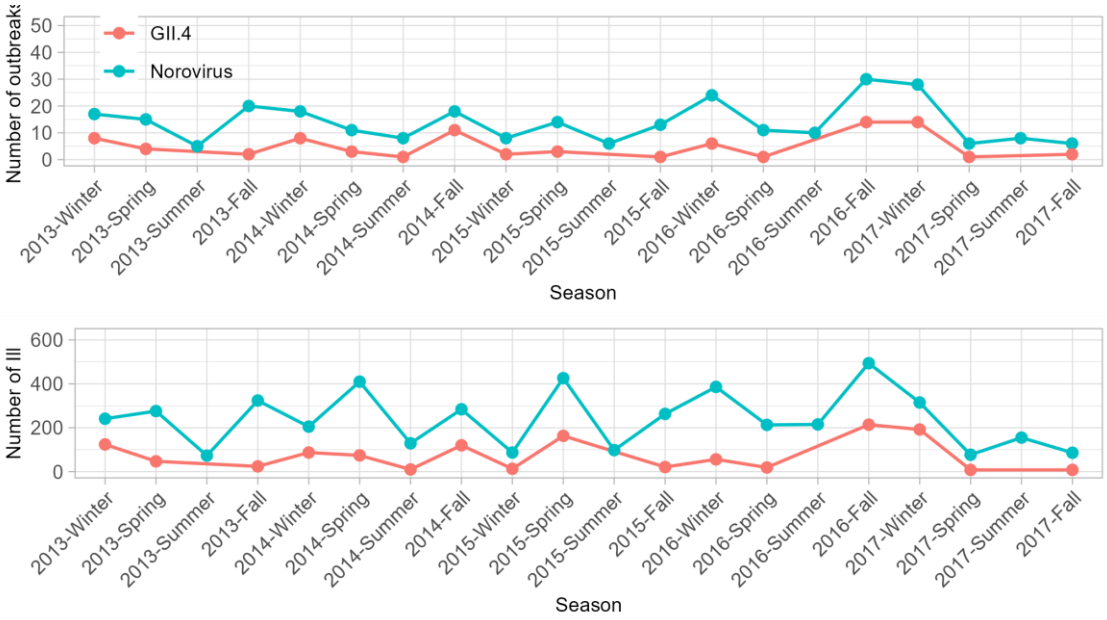

Figure 9. Number of norovirus and GII.4 outbreaks and Ill reported to the NRC since the start of the NRC activity.

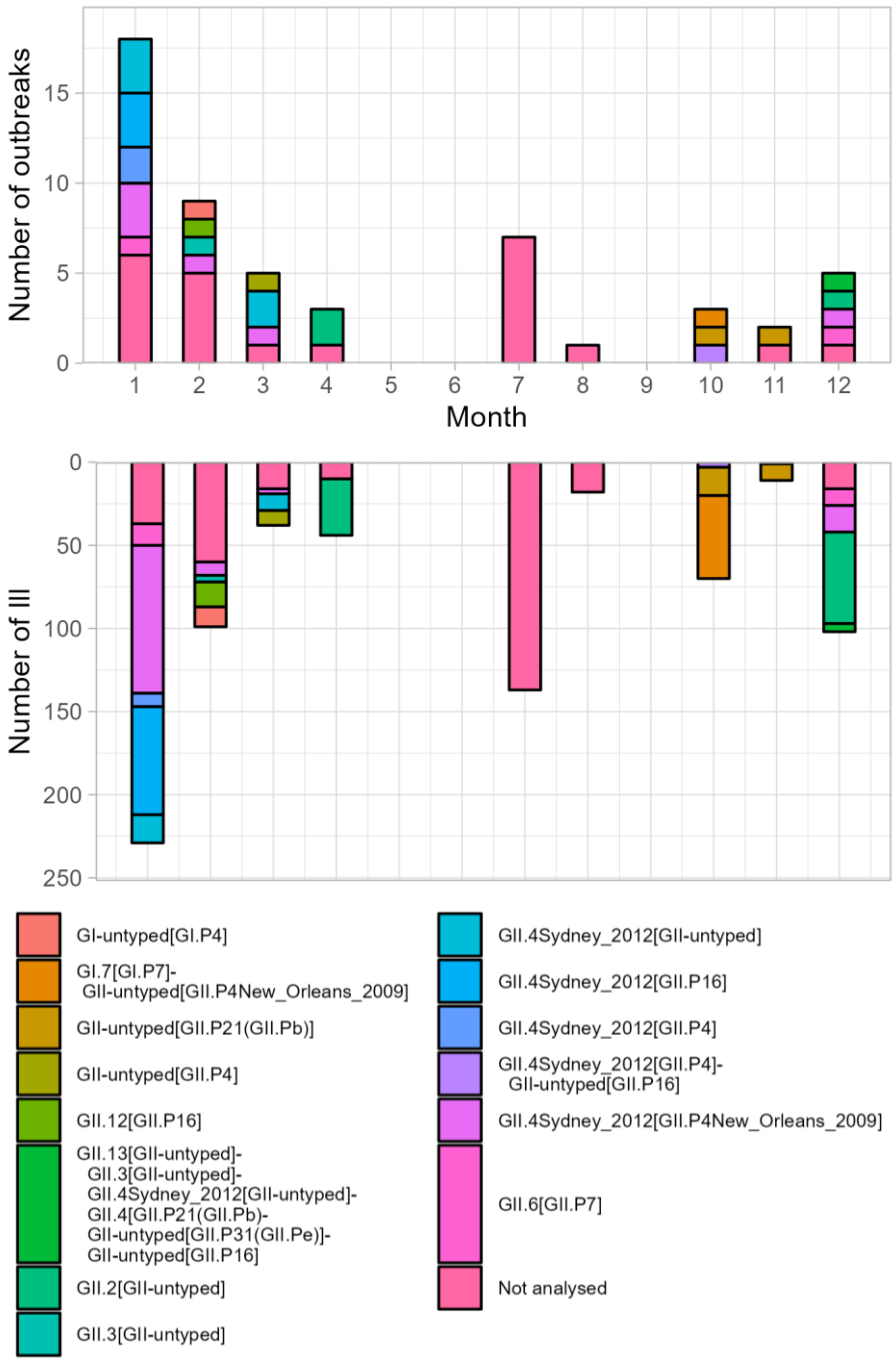

Figure 10. Number of norovirus outbreaks and ill per setting as reported to the NRC in 2017. "Not analysed" comprises all samples that were either not received by the NRC, contained low levels of norovirus, or experienced unsuccessful sequencing.

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.6, P4, P7, P21 and P31 could also be found in these settings. The genotypes more related to foodborne outbreaks were norovirus GI.2, GII.2, GI.3, P2 and P3 (figure 11).

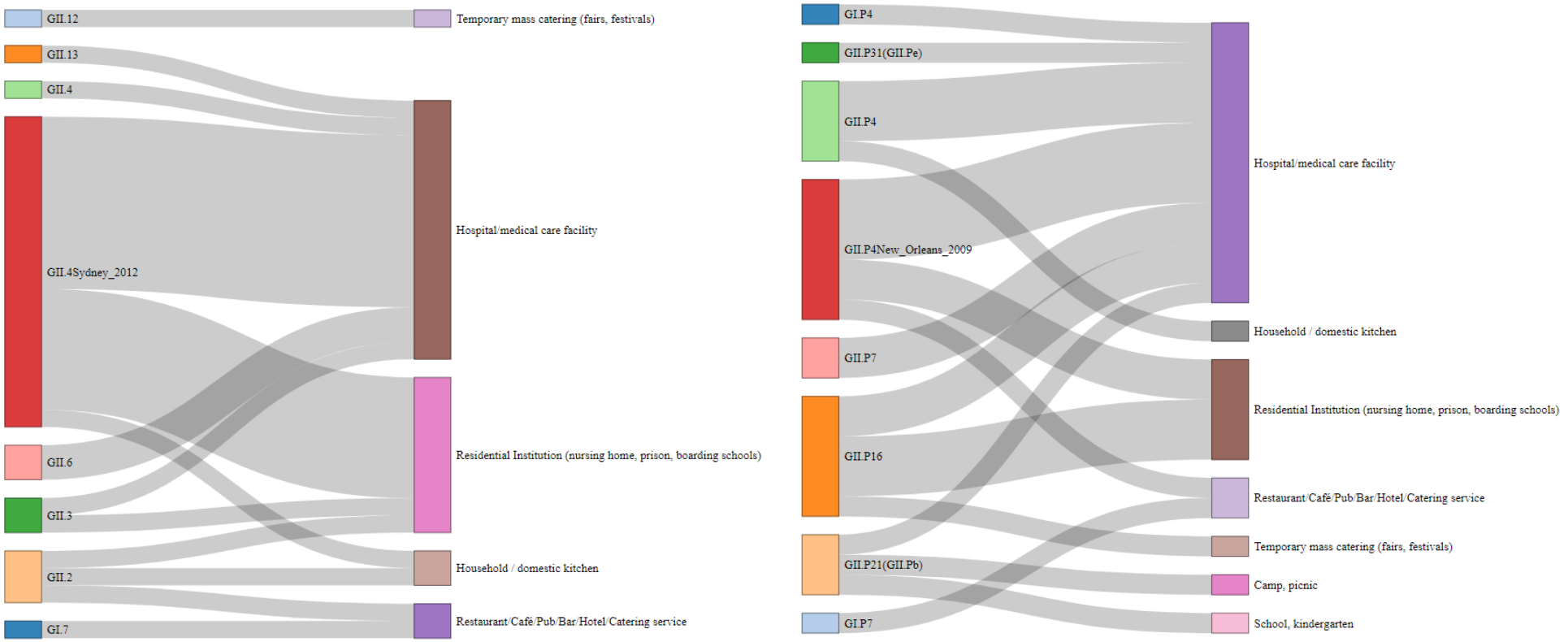

Figure 11. Sankey diagram of the capsid types (left) and polymerase types (right) and their association with the setting of the norovirus outbreaks in 2017.

REFERENCES

- van Beek J, Ambert-Balay K, Botteldoorn N, Eden JS, Fonager J, Hewitt J, Iritani N, Kroneman A, Vennema H, Vinjé J, White PA, Koopmans M; NoroNet. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. Euro Surveill. 2013 Jan 3;18(1):8-9.

- Vinjé J, Hamidjaja RA, Sobsey MD. Development and application of a capsid VP1 (region D) based reverse transcription PCR assay for genotyping of genogroup I and II noroviruses. J Virol Methods. 2004 Mar 15;116(2):109-17.

- Kroneman A, Vennema H, Deforche K, v d Avoort H, Peñaranda S, Oberste MS, Vinjé J, Koopmans M. An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol. 2011 Jun;51(2):121-5.

- Chhabra P, de Graaf M, Parra GI, Chan MC, Green K, Martella V, Wang Q, White PA, Katayama K, Vennema H, Koopmans MPG, Vinjé J. Updated classification of norovirus genogroups and genotypes. J Gen Virol. 2019 Oct;100(10):1393-1406.

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