




Norovirus outbreak in a natural playground: A One Health approach

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Abstract

Norovirus constitutes the most frequently identified infectious cause of disease outbreaks associated with untreated recreational water. When investigating outbreaks related to surface water, a One Health approach is insightful. Historically, there has been a focus on potential contamination of recreational water by bird droppings and a recent publication demonstrating human noroviruses in bird faeces suggested this should be investigated in future water-related norovirus outbreaks. Here, we describe a One Health approach investigating a norovirus outbreak in a natural playground. On social media, a large amount of waterfowl were reported to defecate near these playground premises leading to speculations about their potential involvement. Surface water, as well as human and bird faecal specimens, was tested for human noroviruses. Norovirus was found to be the most likely cause of the outbreak but there was no evidence for transmission via waterfowl. Cases had become known on social media prior to notification to the public health service underscoring the potential of online media as an early warning system. In view of known risk factors, advice was given for future outbreak investigations and natural playground design.

KEYWORDS

birds, faeces, norovirus, One Health, social media, water

1 | INTRODUCTION

In July 2018, the Public Health Service (PHS) received several notifications via a national reporting system for water-quality and water-related health issues from persons who had become sick following water contact in the vicinity of a natural playground. Reports mostly concerned children who, during the previous weekend, had had water contact in a recreational lake as well as

a nearby natural playground which consists of shallow playground water. The outbreak reached the press and fuelled discussion on local social media in which an unusually large number of geese defecating near the playground were discussed among social media users. Outbreak and symptom characteristics, consisting of an acute-onset and relatively swift disappearance of symptoms of diarrhoea and vomiting, were suggestive of an outbreak with norovirus (NoV) which constitutes the most frequently identified

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infectious cause of outbreaks associated with untreated recreational water (de Graaf, Beek, & Koopmans, 2016; Graciaa et al., 2018; Matthews et al., 2012; Rockx et al., 2002).

In the current investigation, a multidisciplinary One Health approach taking into account human, animal and environmental factors was applied to determine the source of the outbreak investigating the role of humans, surface water and waterfowl (World Health Organization, 2017).

2 | MATERIALS AND METHODS

2.1 | Outbreak description

Patient details could be obtained directly because patients had submitted personal notifications to a national reporting system for water-quality and water-related health issues (Meldpunt Water). The PHS was notified of the outbreak on July 4. A timetable demonstrating key events is depicted in Table 1.

To get a more precise overview of outbreak details and proactively screen for further signs of unrest, the PHS monitored public social media via individual queries. On July 4, the day of the notification, a local health-related Facebook-platform posted results of an online request that had started the same day to estimate the total number of sick persons. In this online request, people had been asked to report “the number of persons within their household that had become ill following water exposure in the area” and “to actively tag other ill persons.” People were also asked to actively “report the age of all ill persons” (moderator Facebook-platform, personal communication).

2.2 | Patient faeces

$N = 7$ patients from three families who had submitted personal notifications to the national online reporting system were contacted on July 6 and asked to send in stool specimens for NoV-testing using previously published methods (Sukhrie et al., 2011; van Beek et al., 2017). For specimens that were positive in RT-PCR, a fragment of approximately ~1,000 bp overlapping open reading frames 1 and 2 (ORF1 and ORF2) was sequenced, enabling genotyping of both the polymerase and capsid genes using the NoroNet typing tool (Kroneman et al., 2011). For the phylogenetic analyses, norovirus GI.2

Impacts

- A norovirus outbreak in a natural playground was investigated via a One Health approach testing human faecal, water and bird faecal specimens. Incidental human introduction of norovirus was the most likely cause of the outbreak. Geese were reported to defecate near the outbreak site, leading to speculations about their potential involvement, but there was no evidence for transmission via waterfowl.
- Monitoring of public social media revealed that cases had become known online prior to notification to the Public Health Service: this underscores the potential of online media as an early warning system. Additional insights concerning, potential, sources of contamination and outbreak magnitude were also obtained.
- Rapid sampling and storage of a variety of environmental specimens is useful so that these can later be tested, not only for typical indicator bacteria, but also for viruses.

capsid sequences were downloaded from ncbi, and not more than two identical sequences per year and location were included ($n = 121$). Maximum likelihood trees of the capsid gene (260 bp) were inferred by PHYML 3.0 software using the general time-reversible nucleotide substitution model. The trees were visualized in FIGTREE v1.4.3.

2.3 | Water

Following the first disease notifications, the municipality placed warning signs in the swimming area and ordered rapid routine testing for indicator bacteria of faecal contamination (*Escherichia coli*, enterococci) and cyanobacteria of the swimming water of the recreational lake. During the summer months, water testing of designated large natural swimming areas, including this specific lake, is routinely performed on a biweekly basis in the Netherlands according to the European Bathing Water Directive (EU BWD) (Schets et al., 2018; The European Parliament & the Council of the European Union, 2006). Rapid testing for faecal indicator bacteria and cyanobacteria of the playground water, which unlike the lake is not included in routine screenings, was initiated as well.

Date	Key action
30 June 2018	Outbreak onset (date first cases)
4 July 2018	Online enquiry started following outbreak discussion on Facebook-platform Notification to Public Health Service
5 July 2018	Inspection playground Collection water specimens Fixing water pump playground
6 July 2018	Collection human stool specimens
12 July 2018	Collection bird droppings

TABLE 1 Date of key events

In close collaboration with local governing bodies, the PHS performed a location visit to the playground on July 5 and sampled $n = 6$ playground water specimens (1 ½ litres each) at four different locations which were transported to the laboratory under cold conditions for NoV-testing. Water specimens were concentrated for viral content using polyethylene glycol precipitation (PEG) as previously described (Lewis & Metcalf, 1988; Sima et al., 2011). In brief, 10 ml of 50% PEG 6000 (Sigma-Aldrich) was added to 40 ml of water sample and incubated overnight at 4°C. Subsequently, specimens were centrifuged at 13,500 g for 1.5 hr, from which the pellets were re-suspended in glycine buffer followed by chloroform-butanol (50%/50% by v/v) treatment. Total nucleic acid from each concentrated water specimen was extracted using High Pure RNA Isolation kit (Roche Diagnostics) and subjected to RT-PCR for NoV-detection.

2.4 | Waterfowl faeces

In light of multiple reports, notably on social media where sustained speculations about their potential role in disease transmission had arisen, of a continuous unusually large amounts of geese defecating near the playground water, a proactive second location visit was performed on July 12. During this visit, $n = 6$ faecal specimens were collected from sites directly adjacent to or in the playground water and transported to the laboratory under cold conditions for NoV-testing using previously published methods (Sukhrie et al., 2011; van Beek et al., 2017). Briefly, a faeces

suspension was prepared with 100 mg or 200 μ l faecal specimen in 800 μ l PBS, and after centrifugation (17,000 g), 200 μ l was used for RNA isolation with the High Pure RNA isolation kit (Roche Diagnostics) and subjected to RT-PCR.

3 | RESULTS

3.1 | Outbreak description

The PHS received $n = 21$ case notifications via the national online reporting system. Reported symptoms consisted of vomiting, diarrhoea, headache and fever. Symptom onset was acute, within 0–3 days. Symptoms disappeared within 3 days. Secondary cases were also reported, consistent with an infectious aetiology. The first day of disease onset was June 30.

The online query of the local health-related Facebook-platform yielded a total of at least $n = 100$ sick persons, almost exclusively children (Figure 1, personal communication moderator Facebook-platform, published with permission).

Patients specifically mentioned visits to the natural playground which they suspected to be the likely source. Patient interviewing by the PHS revealed that there had been no food stands at the location, nor were there other indications of food as a common outbreak source.

The location visit on July 5 revealed the playground area schematically depicted in Figure 2. Briefly, a solar-energy-driven water

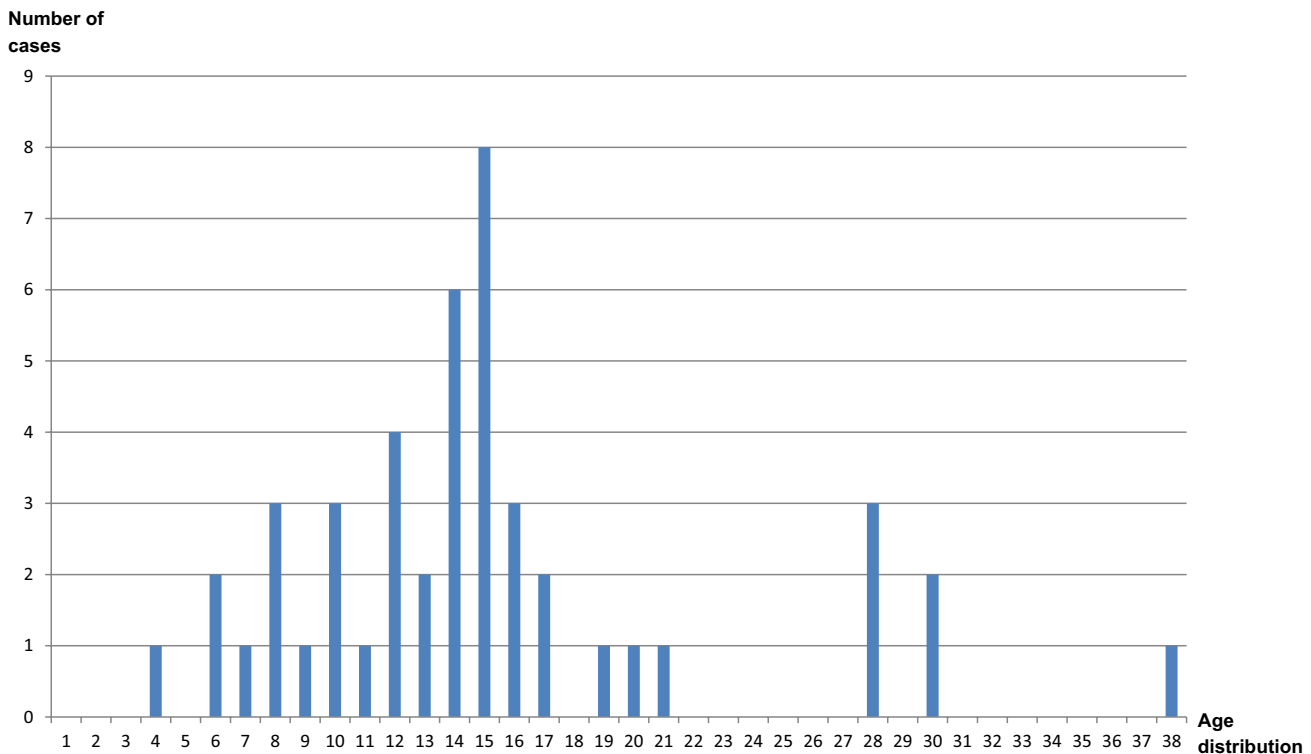


FIGURE 1 Number and age distribution of cases notified via an online social media enquiry. X-axis: age distribution; Y-axis: number of cases (total $n = 101$, $n = 55$ age unknown) (personal communication moderator Facebook-platform, published with permission) [Colour figure can be viewed at wileyonlinelibrary.com]

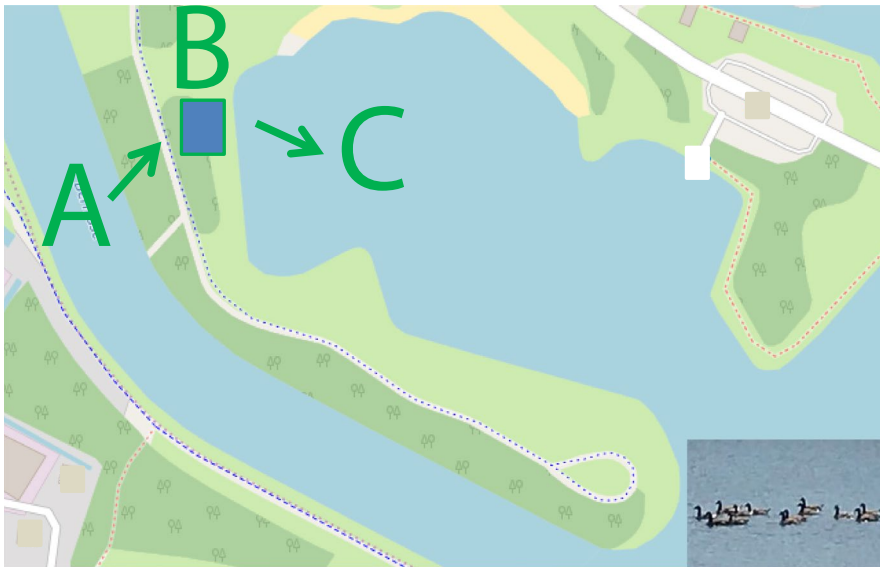


FIGURE 2 Schematic depiction of natural playground area. Water from the nearby river (A) is pumped into a natural playground area (B) from where it flows into the adjacent lake (C). Waterfowl (insert) reside near the playground area. Map: © OpenStreetMap contributors <https://www.openstreetmap.org/copyright> [Colour figure can be viewed at wileyonlinelibrary.com]

pump distributes water from a nearby river (A) into a natural playground area (B) consisting of two metal water dispensers out of which water flows into a shallow natural playground basin, where mud water is formed, and then into the adjacent lake (C). There were waste bins and, at a nearby beach, public toilets. There were no food trucks. The location visit demonstrated that the water pump designed to refresh the playground basin with fresh river water had broken down. Because of this, a situation with non-refreshed shallow playground water had existed for an unknown duration. The pump was repaired directly after the outbreak, on July 5, and water was refreshed.

3.2 | Patient faeces

$n = 4$ patients out of two different families returned stool specimens. 4/4 specimens were positive for NoV GI. One stool specimen, additionally, tested positive for enterovirus and one for adenovirus genotype non-40/41. All specimens were sequenced to determine the infecting GI genotype, and 4/4 specimens contained NoV genotype GI.P2-GI.2 as determined by the NoroNet typing tool and phylogenetic analysis (Figure 3). Norovirus GI-related outbreaks are less frequent compared to GII outbreaks, but GI.P2-GI.2 has previously been detected in outbreaks in the Netherlands (van Beek et al., 2018).

3.3 | Water

Test values for *Escherichia coli*, enterococci and cyanobacteria on water specimens from the recreational lake as well as from the refreshed playground water specimens were below cut-off values set by the EU BWD (The European Parliament & the Council of the European Union, 2006). 6/6 playground water specimens tested negative for NoVs GI and GII.

3.4 | Waterfowl faeces

During the second location visit, fuelled by reports of large numbers of defecating geese, significant quantities of thin herbivorous faecal specimens, most likely geese faeces, were spotted near the playground water. 6/6 tested faecal specimens were negative for NoVs GI and GII.

4 | DISCUSSION

This outbreak investigation illustrates a number of important considerations.

Norovirus, incidentally introduced by a child, or multiple children, recreating in the playground water, was the most likely cause of the outbreak. Preventive measures, including installing a water pump, public toilets and waste bins, were taken when designing the playground, and it is difficult to assess the exact role of a defunct pump, as norovirus is highly infectious in small quantities. However, nearly all risk factors for increased faecal contamination were present in this outbreak setting, that is shallow, poorly circulating water, frequented by young children and animals (Gracia et al., 2018).

In accordance with the 10-steps of outbreak investigation, the outbreak was structurally investigated and followed a One Health approach (Prikazsky, V. (Ed.)).

Monitoring of public social media proved of added value in the outbreak investigation. Interestingly, cases had already become known via social media, enabling an online investigation to be started, before notification to the PHS (Table 1). Although exact reliability can be an issue, previous publications have demonstrated the value of online monitoring strategies as early warning systems (Ginsberg et al., 2009; Liu et al., 2017; van de Belt et al., 2018).

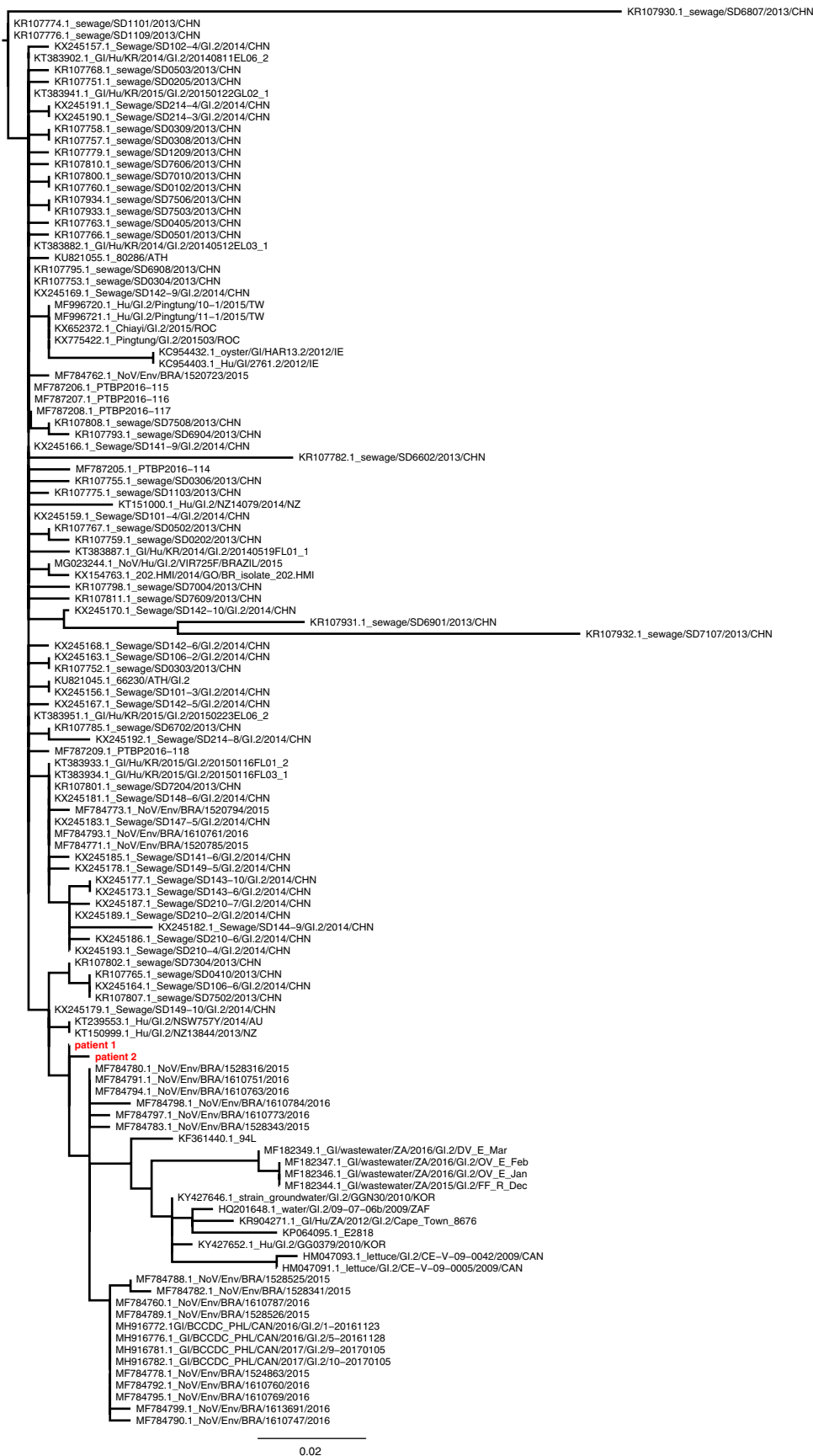


FIGURE 3 Phylogenetic tree demonstrating norovirus interrelatedness. Maximum-likelihood trees of ORF 2 were inferred by PHYLX 3.0 software using the general time reversible nucleotide substitution model. Scale bars indicate nucleotide substitutions per site. The sequences of two outbreak samples are depicted in red [Colour figure can be viewed at wileyonlinelibrary.com]

From a One Health perspective, an outbreak related to surface water warrants a multidisciplinary approach investigating human, environmental and animal factors (Graciaa et al., 2018; Summa, Henttonen, & Maunula, 2018). As surface water is not routinely tested for NoVs, water specimens were only included for NoV-testing following notifications of disease when the playground water had already been refreshed. In future outbreak settings, it is advisable to obtain water specimens directly following disease outbreaks near recreational water. These can be stored under cold conditions and tested at a later moment. The same holds true for other environmental specimens, such as subsurface sand specimens, which can be positive for NoV while the water is negative (Schets et al., 2018).

In light of a recent publication on detection of human norovirus (HuNoV) in bird faeces in non-outbreak settings, it is of importance that, in our case, bird faecal specimens underwent testing for HuNoV-testing as part of the outbreak investigation and were found to be negative (Summa et al., 2018). There has been a lot of historical attention on the disease-transmitting potential of bird faeces with a focus on bacteria and parasites (Benskin, Wilson, Jones, & Hartley, 2009; Elmer, Berg, Lerner, Waldenstrom, & Hessel, 2017; Gorham & Lee, 2016; Graczyk, Majewska, & Schwab, 2008; Meerburg, Koene, & Kleijn, 2011). In this respect, however, a recent review found evidence suggesting HuNoV-transmission could in principle rather be directed from humans or the environment towards animals (Villabruna, Koopmans, & de Graaf, 2019).

Overall, our findings may serve to stress the fact that routine water testing for faecal bacteria and harmful algal blooms are not always a good indicator of viral-contamination which is in line with previous publications (Rose, Mullinax, Singh, Yates, & Gerba, 1987; Sinclair, Jones, & Gerba, 2009). It is advisable to collect a variety of environmental specimens, such as water specimens, subsurface sand specimens and animal stool, as soon as possible following an outbreak. Non-outbreak-related routine collection can increase knowledge on the background presence and relevance of norovirus.

In an era in which all sorts of natural recreation are gaining popularity, it is prudent that clear guidelines are developed describing minimal requirements for recreational areas. In this case, the playground was well-designed and thoroughly constructed but a pump that was crucial for water refreshment went defunct. In general, though, measures should incorporate minimal hygiene facilities, such as a sufficient amount of garbage containers, simple shower, toilet, and diaper-changing facilities and a system avoiding poorly circulating shallow water. The public should be advised to avoid recreational activities in natural water when sick.

Ultimately, in accordance with the One Health approach described here, it should also be realized that these leisure areas are situated in the natural habitat of animals, keeping them in mind in our designs and interventions.

CONFLICT OF INTEREST

None.

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