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Short Communication

Large norovirus GII.17 outbreak in a school in Northern Italy: A novel emerging sublineage within an emerging genotype?

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ABSTRACT

Norovirus (NoV) is one of the leading causes of acute gastroenteritis worldwide, being responsible for millions of cases each year. We report a large NoV outbreak occurred in a Northern Italy school complex, and the genomic characterization of the outbreak strain. Stool samples from 105 subjects involved in the outbreak were collected and tested for enteric pathogens by real-time RT-PCR. The infecting NoV strain was genotyped by whole genome sequencing (WGS). Finally, a phylogenetic analysis was carried out including all whole genomes available online for the same genotype. NoV GII genogroup was detectable in 85 of 105 (81%) tested stool samples. WGS performed on 32 (32/85, 37.6%) positive samples evidenced the infecting NoV strain as belonging toGII.17[P17] genotype. The phylogenetic analysis showed that its sequences clustered with recent GII.17[P17] isolates, mostly from Europe, while they formed a separated subclade with distinct mutational patterns. Our analysis confirmed all sequenced isolates belonged to the same transmission event.

The detected genotype and the mutational pattern of the outbreak strain further support the emergent role of GII.17[P17] among NoV strains worldwide and the need of its close epidemiological monitoring.

Introduction

Human noroviruses (NoV) are small non enveloped single-stranded RNA viruses that belong to the family *Caliciviridae*. They are classified into at least 10 genogroups (G), of which viruses from GI and GII cause the vast majority of NoV infections in humans [1].

NoV are endemic worldwide, causing gastroenteritis with symptoms including stomach cramps, diarrhea and vomiting, which may last up to 4 d [1]. Severe NoV disease typically affects infants, the elderly and immunocompromised individuals, and may require hospitalization. NoV are commonly transmitted via the fecal–oral route and can be transmitted through food, water, air, person-to-person contact, or the environment [1].

We reported a human NoV gastroenteritis outbreak in a Northern Italy school complex in March 2025 involving 168 symptomatic subjects among nursery and primary school students, teachers, school canteen staff members and parents. On 04/03/2025, several children presented at the local emergency department (ED) suggesting a possible foodborne toxic infection at a school complex. Epidemiological investigations began at the affected school the following day, and the cases were monitored.

Material and methods

According to the Italian law on preventive healthcare, diagnostic investigations that are necessary for proper public health man-

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agement, but do not directly affect patient care (e.g., genotyping), do not require mandatory written informed consent. However, subjects have been informed of the investigations in accordance with the legislative decree (DGR 3698 del 20/12/2024). The epidemiological analysis of the outbreak included 305 potentially exposed subjects, that is, those having consumed at least one meal at the school canteen within the 2 d before the first case. Cases were considered as those presenting or reporting at least one of the following: vomiting, abdominal pain, diarrhea, fever, nausea, and headache.

NoV detection and genogroup assignment was performed by real-time RT-PCR Xpert® Norovirus (Cepheid, Sunnyvale, CA, USA) on stool samples of 105 symptomatic subjects, that is, those presenting at the ED or successfully contacted during the following outbreak investigation.

Thirty-two samples were selected for whole genome sequencing (WGS), to be representative of all the categories of subjects involved in the outbreak. Viral RNA from stool samples was manually extracted with the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. RNA was eluted in 50 µL of water. Specimens and RNA were stored at −80°C until use. Full genomes were obtained with NextSeq Illumina platform. Library preparation was conducted with NextSeq RNA Prep with Enrichment, Viral surveillance Panel kit (Illumina, San Diego,CA,USA) [2]. Libraries were normalized and pooled for sequencing using a 2 × 150 cycle paired-end sequencing protocol. Consensus sequences were generated mapping FASTQ files to a reference sequence (LC369244.1) and with *de novo* assembly with Geneious Prime software v. 11.1 (https://www.geneious.com/). Two samples were subsequently excluded from the analysis for inadequate coverage. The average vertical sequencing coverage was found to be 115,684X for the remaining 30 samples (minimum: 5.8X, maximum: 953,898.7X).

NoV genotype was determined using Norovirus Typing Tool Version 2.0 (https://mpf.rivm.nl/mpf/typingtool/norovirus/) and Human Calicivirus typing tool(https://calicivirustypingtool.cdc.gov).

The whole genomes (approximately 7.3 kb) were aligned and edited using BioEdit v. 7.2.6.1 program (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Phylogenetic models GTR+F+I+G4and GTR+F+R2were estimated and selected using the ModelFinder implemented in IQ-TREE v.2(http://www.iqtree.org/). The reliability of clades was established based on bootstrap values of internal nodes ≥70% (after 1000 replications). Maximum Likelihood dating was obtained by the least square dating method (LSD2) integrated in IQ-TREE with 100 replicates to obtain confidence intervals in time of the most recent common ancestor (tMRCA) estimates. Nucleotide sequences were submitted to the GenBank database under accession numbers: PV765564 - PV765590, PV902474 (Supplementary Table 1). Two datasets were built, the first included all WGS of GII.17[P17] genotype available online. Thus, 168 whole genome sequences of NoV belonging to the same subgroup and genotype, available on 2025.06.24, were retrieved. The complete list of references is reported in S1. Further investigations were conducted using only the European sequences.

Results

Among the 263 potentially exposed students, 156 were symptomatic with a global attack rate of 59.3%; more in details, the attack rate was 24.4% (10/41), 65.8% (146/222) and 28.6% (12/42), among nursery school students, primary school students, and teachers and canteen staff members, respectively.

Among symptomatic subjects (55.1%, 168/305) 82 (48.8%) were males and 86 (55.1%) females, including 156 students (92.9%, median age 9, IQR:8-10), 9 teachers (5.3%) and 3 (1.9%) canteen staff members (median age 52, IQR:30-57). Main symptoms observed were vomiting (79.2%), abdominal pain (60.7%), diarrhea (48.2%), fever (36.9%), nausea (33.9%), and headache (11.3%). Only one child was admitted for 48 h to the pediatric ward.

All food samples of served meals tested negative for NoV by molecular methods, thus ruling out a possible foodborne cause. Among human tested samples 81% (85/105) resulted positive for NoV GII, including the samples of three parents of symptomatic students to be considered secondary intra-family cases. WGS assigned all viral isolates to GII.17[P17] genotype.

The phylogenetic trees including international (Figure 1A) and European (Figure 1B) sequences showed that all the sequences of the outbreak described in this paper are included in a single, distinct clade with high statistical support (bootstrap 100), confirming that they belong to the same outbreak (subclade 1b).

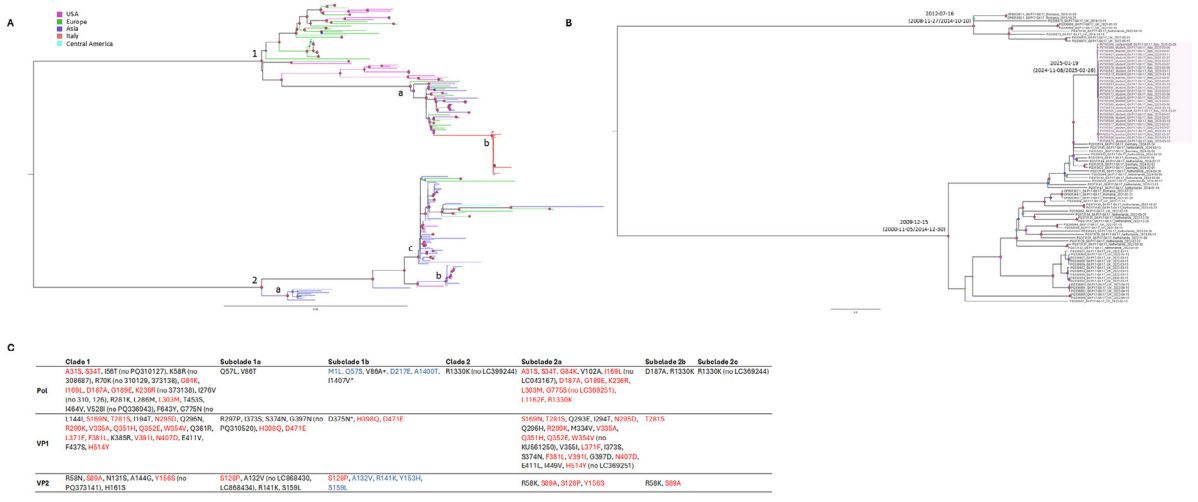

Figure 1. Maximum likelihood tree including all available NoV whole genome GII.17[P17] sequences (Part A) and dated tree European NoV whole genome GII.17[P17] sequences (Part B). Node sizes are proportional to bootstrap support-max size = 100%. Part A: Branches are colored based on geographical origin of samples as reported in the legend. Main clades are indicated with numbers and letters. Part B: Estimated dates are reported for main clades. Clade containing the Italian strains is highlighted in pink. Part C: table summarizing the list of amino acid mutations characterizing the main identified clades of the NoV sequences. Mutations observed in both clades 1 and 2 are highlighted in red. Mutations exclusively of Italians are highlighted in blue.
+Shared with 41/55 sequences of Clade 1; ^Shared with 37/55 sequences of Clade 1; *Shared with 10/45 sequences of Subclade 2c.

In Figure 1A, two main clades were evident, the former including more recent strains mostly from Europe (clade 1), and the latter encompassing the majority of sequences from South-East Asia (clade 2) (Figure 1A). Within clade 1, the sequences of our outbreak formed a large supported (bootstrap 82)cluster. In the dated tree (Figure 1B) previous observed clade 1 dated back to December 2009 and clade of Italian strains dated back to January 2025. European strains clustering in Figure 1A with international strains formed a separate clade in Figure 1B dating back to July 2012.

Of note, the Italian sequences involved in the presented outbreak had numerous distinctive mutations in all analyzed genes (pol, VP1 and VP2) compared to the sequences of complete genomes present in the databases (Figure 1C), further confirming their very recent origin. Some of the observed mutations were characteristic of the European strains, while others were also observed in older sequences from Asia.

Discussion

Our analysis reveals that all sequenced isolates within the described outbreak belong to the same transmission event. The infecting NoV belong to genogroup II and genotype GII.17[P17], an emerging genotype since 2014 in various countries, more recently observed in large outbreaks in Europe and North America [3–5] but, to the best of knowledge, never reported on such a large scale in Italy to date [6,7]. However, the distinct mutational characteristics of Italian strains, which differ from those of the isolates of the same genotype available in online databases, suggest that they could represent a new emerging sublineage within the GII.17[P17] genotype. Unfortunately, the lack of sequences from similar recent outbreaks do not allow to infer whether the observed differences are only geographically-related or they characterize an emerging sublineage with a broader epidemiological impact.

Due to its high circulation and remarkable diffusibility, NoV must be continuously monitored in its evolution to provide an epidemiological overview and lay the groundwork for possible prophylactic measures [7]. Close monitoring of NoV is lacking at the human clinical level in Italy, as evidenced by the limited data reported in the literature and in online sequence databases [8]. Our data highlight the importance of multi-step approaches to successfully investigate NoV outbreaks and emphasize how thorough environmental health and laboratory investigations must be supported by epidemiological findings from a one-health perspective, to be important also on a larger epidemiological scale.

Author Contributions

Conceptualization, **F.N., A.L.** and **N.M.**; Methodology, **A.P., G.Z, C.M.G.A., M.B, M.P.C, G.D.C, S.V.C.Z, G.B,**; Formal Analysis, **F.N.** and **A.L.**; Investigation, **A.D, G.B.** and **D.C.**; Resources, **FN** and **N.M.**; Data Curation, **F.N., A.L.**; Writing Original Draft Preparation, **F.N., A.L,** and **N.M.**; Writing Review & Editing, **S.V.C.Z, G.B** and **G.A.**; Visualization, **F.N., A.L.** and **G.A.**; Supervision, **F.N, A.L.** and **N.M.**; Project Administration, **N.M.** and **F.N.**; Funding Acquisition, **F.N.** All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.108185.

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